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► **To cite this version:**

Yves Vandecan, Emanuele Biondi, Ralf Blossey. Core-oscillator model of *Caulobacter crescentus*. *Physical Review E: Statistical, Nonlinear, and Soft Matter Physics*, 2016, 93 (6), 10.1103/PhysRevE.93.062413 . hal-01440773

HAL Id: hal-01440773

<https://hal.science/hal-01440773>

Submitted on 18 May 2021

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Core-oscillator model of *Caulobacter crescentus*Yves Vandecan,¹ Emanuele Biondi,² and Ralf Blossey¹¹*Université de Lille 1, CNRS, UGSF UMR 8576, 59000 Lille, France*²*Aix-Marseille Université, CNRS, LCB UMR 7283, 13009 Marseille, France*

(Received 27 January 2016; revised manuscript received 30 March 2016; published 22 June 2016)

The gram-negative bacterium *Caulobacter crescentus* is a powerful model organism for studies of bacterial cell cycle regulation. Although the major regulators and their connections in *Caulobacter* have been identified, it still is a challenge to properly understand the dynamics of its circuitry which accounts for both cell cycle progression and arrest. We show that the key decision module in *Caulobacter* is built from a limit cycle oscillator which controls the DNA replication program. The effect of an induced cell cycle arrest is demonstrated to be a key feature to classify the underlying dynamics.

DOI: [10.1103/PhysRevE.93.062413](https://doi.org/10.1103/PhysRevE.93.062413)**I. INTRODUCTION**

The notion of the cell cycle encompasses the complete set of molecular and structural events that take place in a cell leading to its division and duplication, ending up in two daughter cells [1]. For both eukaryotic and prokaryotic cells, advances in our understanding of the molecular interactions driving these transitions has led to the possibility to formulate advanced mathematical models which cover many fundamental aspects of the cell cycle and allow for a quantitative understanding of the involved processes [2,3]. In this paper we are concerned with the cell cycle of a bacterium which has a complex lifestyle, the alphaproteobacterium *Caulobacter crescentus*, for short *Caulobacter*. *Caulobacter* differentiates between a mobile swarmer cell which moves with a flagellum in its aqueous environment and a stationary stalker cell which anchors at a surface. The bacterium thus has an asymmetric cell division which is a sequence of swarmer to stalked differentiation (G1-S transition), the DNA replication phase (S) together with the development of the early pre-divisional cell, and finally the phase of cellular division, i.e., the splitting of the late pre-divisional cell into a daughter stalked and swarmer cell (G2), as illustrated in Fig. 1.

The cell cycle of *Caulobacter* has already seen a number of modeling attempts [4–9], similar in spirit to earlier work performed on the eukaryotic cell cycle. Although a simple organism, a bacterial cell possesses multiple layers of cell cycle regulation that have evolved in order to respond to its life style. Understanding the complexity of this circuit is a difficult task, however some components of this network are clearly more important than others and therefore more likely to be conserved across different bacterial species. In a recent combined experimental and modeling study two of the previously assumed key regulators—part of the extended models of the *Caulobacter* cell cycle—turned out to be dispensable [10], i.e., the cells retain viability after deletion of the corresponding genes. Finding the minimal components able to guarantee cell cycle oscillations gives scientists the opportunity to first simplify the complexity of biological networks and more importantly to focus on important components when investigating other bacterial species. Starting from a minimal model, possibly applicable to different species, may thus allow a modular approach in which new modules can be added to the minimal core circuit. In this paper, we try to implement this idea by

focusing on the replication step (G1-S) in *Caulobacter* as it lays the basis for cell division.

II. MODEL CONSTRUCTION

The two key regulators of replication are firstly the master regulator of *Caulobacter*, the protein CtrA, which in fact is implied in a large number of regulatory mechanisms [11–15]. In the context of DNA replication, its task is to repress the origin of replication by blocking the action of its antagonist, DnaA [16]. DnaA is required to initiate DNA replication, but it is also involved in activation of transcription of several cell cycle genes [17]. DnaA, in turn, also activates the transcription of the gene encoding for CtrA. The activity of CtrA is modulated by the protein DivK in a similar fashion: it both inactivates CtrA while it is activated by CtrA. Both CtrA and DivK are the endpoints of phosphotransfer modules: DivL-CckA-CtrA and DivJ-PleC-DivK [18–21]. Activation and inactivation of the molecules is thus modulated by switching between phosphorylated and dephosphorylated forms, and their respective spatial localization in the predivisional cell. In order to treat all these aspects, as well as the ultimate cell division, obviously more complex models are needed and have, in part, already been developed, as referred to above.

Focussing on the main architecture of the regulatory modules, we summarize the interactions retained in our model in Fig. 2 which graphically shows that the key activation step of replication is the coupling between CtrA and DnaA, and that CtrA in turn is implied in the second interaction pair, CtrA–DivK. The question is to understand how the three proteins together organize and control replication.

Turning to the mathematical formulation, we make use of the approach developed in [22,23] which is best suited for the analysis of core networks, in particular when considering also cell cycle arrest. Denoting the proteins by $x_i, i = 1, \dots, 3$ we have (CtrA, DivK, DnaA) $\equiv (x_1, x_2, x_3)$. Each of the proteins fulfills a dynamic equation

$$\tau_i \frac{dx_i}{dt} = \left(s_i + \sum_j a_{ij} x_j^{n_j} \right) (1 - x_i) - \left(d_i + \sum_j b_{ij} x_j^{n_j} \right) x_i, \quad (1)$$

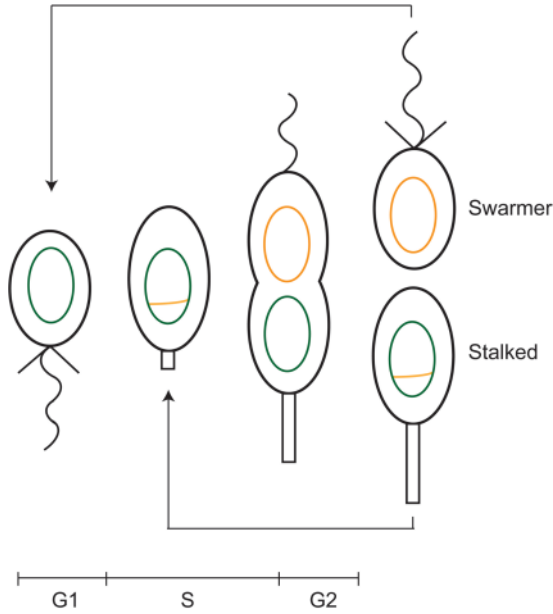


FIG. 1. Cell cycle progression in *Caulobacter*: the swarmer cell undergoes a transition to an anchored cell (G1 → S); the DNA replicates in S-phase, cell division leads to daughter stalked and swarmer cells (G2).

in which the τ_i are relaxation constants, the s_i basal activation constants for each protein, and the a_{ij} and b_{ij} are activation and inactivation matrices that govern the interactions depicted in Fig. 2. The diagonal elements a_{ii} are the self-interactions of the proteins, shown as curved arrows in Fig. 2. The cross-diagonal parameters in both the activation and inactivation matrices describe the couplings between the different proteins. Finally, in the model, the remaining parameters d_i are the basal degradation rates of each of the proteins; in absence of the constant terms and the coupling terms, protein concentrations decay exponentially like $x_i \sim \exp -(d_i/\tau_i)t$. The model can be derived from basic models of gene regulation, as detailed in the Appendices of [22,23]. In applying the model to *Caulobacter*, we note that dimerization is experimentally verified in all three proteins, CtrA [24], DivK [4,25,26], and DnaA [27], so that we can fix the value of the Hill coefficient $n_j = 2$ for all j .

According to the wiring of Fig. 2, for *Caulobacter* we have the following activation and inactivation matrices a_{ij} and b_{ij} , respectively, containing the five activating and two inactivating

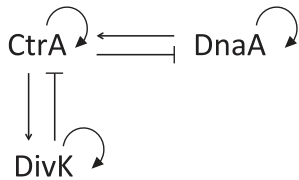


FIG. 2. (a) The minimal regulatory circuit of *Caulobacter crescentus* is a system of three proteins CtrA, DivK, and DnaA, connected by synergistic and opposing interactions in such a manner as to form a *coupled limit-cycle oscillator*, i.e., CtrA-DivK and CtrA-DnaA. Arrows correspond to activation, turnstiles to inactivation, e.g., CtrA activates DivK, while DivK inactivates CtrA.

interactions as their entries:

$$a_{ij} = \begin{pmatrix} a_{11} & 0 & a_{13} \\ a_{21} & a_{22} & 0 \\ 0 & 0 & a_{33} \end{pmatrix} \quad b_{ij} = \begin{pmatrix} 0 & b_{12} & 0 \\ 0 & 0 & 0 \\ b_{31} & 0 & 0 \end{pmatrix}, \quad (2)$$

such that the model can be explicitly written as

$$\begin{aligned} \tau_1 \frac{dx_1}{dt} &= (s_1 + a_{11}x_1^2 + a_{13}x_3^2)(1 - x_1) - (d_1 + b_{12}x_2^2)x_1, \\ \tau_2 \frac{dx_2}{dt} &= (s_2 + a_{21}x_1^2 + a_{22}x_2^2)(1 - x_2) - d_2x_2, \\ \tau_3 \frac{dx_3}{dt} &= (s_3 + a_{33}x_3^2)(1 - x_3) - (d_3 + b_{31}x_1^2)x_3. \end{aligned} \quad (3)$$

III. MODEL PARAMETRIZATION

In order to determine the parameter values of the model, we need to gather information from experiment. Qualitatively, DnaA and CtrA change during the cell cycle progression in an opposing fashion, with the DnaA levels increasing when CtrA is degraded and disappearing in predivisitional cells when CtrA is synthesized again by the GcrA-dependent transcriptional activation [16]. The total DivK level (unphosphorylated and phosphorylated) is fairly stable over the cell cycle with the concentration of the protein proportional to the cellular volume [20]. Protein concentrations were thus estimated as relative amounts (%) with respect to the time 0 concentration, and CtrA, DnaA, and DivK data were extracted from previous studies [16,20]. These data are collected in Table I and also shown in Fig. 3. We note that the extraction of the experimental data for DivK must be done by reading from a gel, with obvious difficulties as the concentrations of total DivK do not vary much. Our reading is similar to what was done in [9].

We now turn to the parametrization of our model, taking the experimental results as our point of reference. Figure 3(a) and (b) shows the time traces for two different parametrizations of the system (3), with the parameters summarized in Table II. We call these parametrizations *weak* and *strong coupling*, respectively, whereby these terms shall refer to the two distinct dynamics we observe. The notions ‘weak’ and ‘strong’ were chosen in order to qualify that in the former case, the amplitude variation of the DivK is minor, hence weakly coupled to the other two regulators, while it is pronounced in the latter case which is correspondingly called ‘strong coupling’.

In Fig. 3(a), DivK is only varying weakly, and the fit to the experimental data is quite good with respect to both DnaA

TABLE I. Experimental data as extracted from [16] (CtrA, DnaA) and [20] (DivK). For the discussion, see text.

Time/Min	CtrA	DivK	DnaA
0	1.0	1.0	1.0
20	0.0	1.0	2.22
40	0.0	1.05	2.0
60	0.0	1.05	0.67
80	0.63	1.25	0.33
100	1.22	1.25	0.22
120	1.06	1.30	0.89

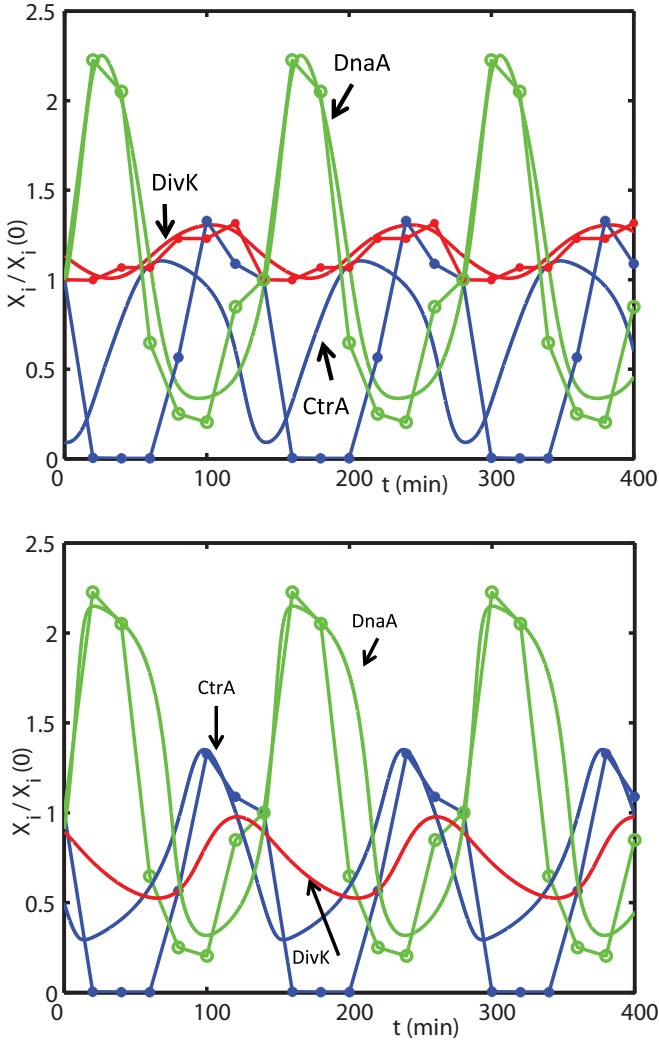


FIG. 3. Time traces of the cell cycle in *Caulobacter*, for CtrA (blue/dark grey), DnaA (green/light grey), and DivK (red/medium grey) for the weakly coupled case (top) and the strongly coupled case (bottom). Lines are also labeled for better readability. Smooth lines are computed, the straight lines connect the experimental data points from Table I.

and DivK, but fairly poor with respect to CtrA. In particular, we note that phasing is not well respected, as the maximum of CtrA appears before the minimum in DnaA. In the strong coupling case of Fig. 3(b) we see that, on the contrary, DnaA and CtrA are well phased with the dynamics of DivK being considerably more pronounced than the experimental data show. One can therefore conclude that we must not simply interpret DivK as just the total component, but rather an

active fraction of its total concentration which is involved in controlling the dynamics of CtrA. In particular, we note that it is due to this effect that the proper phasing of the two motifs is achieved.

IV. CELL CYCLE ARREST

The two parametrizations of the model show different qualitative and quantitative behaviors which also have consequences beyond the normal cell behavior on which these parametrizations have been based. We show this by considering cell cycle arrest. A recent experimental study has provided evidence that proteotoxic stress via a depletion of the Hsp70 chaperone, DnaK, either through genetic manipulation or heat shock to which *Caulobacter* is exposed, acts on DnaA by promoting its rapid degradation [28]. This mechanism can be captured in our model by increasing the degradation parameter of DnaA, d_3^* . We have therefore studied the behavior of the model that emerges when d_3^* is taken as a bifurcation parameter. We determined the fixed-points of the system (3) and studied the local and global stability of the solutions, see also the Supplemental Material.

The two resulting one-parameter bifurcation diagrams are shown in Fig. 4. Figure 4(a) corresponds to the weak coupling case. We find that at a critical value of $d_{3,crit}^* < 1.15$ the system displays a supercritical Hopf bifurcation. Looking at the transition from lowering the value of d_3^* , the stable (arrest) state becomes unstable with respect to a limit cycle in a continuous fashion.

The situation we encounter in the strong coupling case, shown in Fig. 4(b), is more complex as we can identify four regimes which are again more easy to explain when considering the lowering of d_3^* . For $d_3^* > 1.163$, the system has two unstable and one stable fixed-point which is the lowest of these branches. At a critical value $d_{3,crit}^* = 1.163$, it becomes unstable with respect to a limit cycle again via a supercritical Hopf bifurcation. This limit cycle has a very small width and remains stable only within a small interval until $d_3^* = 1.158$ when it collapses with respect to the above-discussed *Caulobacter* limit cycle via a saddle-node bifurcation of limit cycles (SNLC). This case is akin to the scenario SNLC₁ discussed in [23]. Until $d_3^* = 1.163$, the dynamics is still influenced by the former stable fixed-point around which the trajectory continue to spiral. Details of the transitions in the oscillation profiles are resolved in the Supplemental figures S2 and S3 [29].

Pfeuty rejects SNLC scenarios on general grounds for biological systems as there is no clear mechanism available to rationalize the transition between two different limit cycles with different amplitudes over an extended range of parameters. In our case the transition sequence occurs over a

TABLE II. Model parametrization for the ‘weak coupling’ (wk) and ‘strong coupling’ (str) cases. The basal degradation constants have all been put equal to 1 in the comparison to the experimental data. The value of a_{22} being either a small number or zero has no effect on the dynamics.

	a_{11}	a_{13}	a_{21}	a_{22}	a_{33}	b_{12}	b_{31}	τ_1	τ_2	τ_3	s_1	s_2	s_3
wk	4	0.3	0.5	0	5	1	3.6	0.2	3	0.42	0.006	0.1	0.14
str	6	0.3	2	0	4	10	3	0.44	4	0.1	0.009	0	0.17

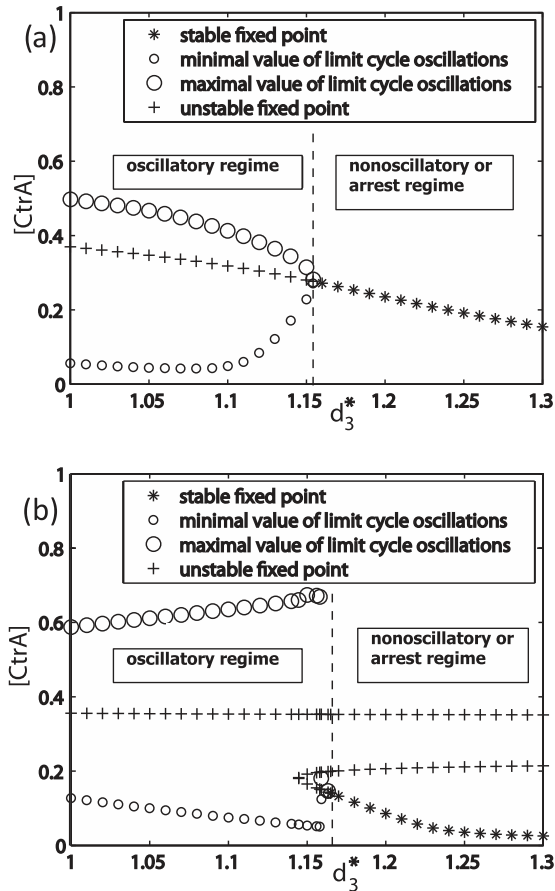


FIG. 4. The two cell cycle arrest scenarios in the weak coupling (a) and strong coupling (b) cases. In case a) the limit cycle shrinks upon approach to the critical value of the degradation constant of DnaA and the transition arises in a continuous fashion. In the strong coupling case b), the limit cycle first expands while an additional branch of unstable fixed points appears. The transition progresses in three steps: limit cycle \rightarrow limit cycle \rightarrow arrest state.

very small interval of the parameter d_3^* so that, even if it existed, it might be easily missed in experiment. The *global* difference between the two scenarios should, however, be visible in

corresponding experiments: in the weak-coupling case the amplitude of the limit cycle oscillations shrinks continuously when the critical values of cell cycle arrest is approached, while in the strong coupling case, a limit cycle of finite width for $d_3^* \ll d_{3,crit}^*$ appears to break down abruptly to the arrest state for $d_3^* \gg d_{3,crit}^*$. In terms of the width of the limit cycle, even the trend upon the approach of $d_{3,crit}^*$ between the two cases is opposite: a shrinking limit cycle in the weak coupling case vs an expanding limit cycle in the strong coupling case. In this latter case the transition would thus not look much different than in the SNIC-SH transitions discussed in detail by Pfeuty [23].

V. CONCLUSIONS

We have shown that the replication step of the cell cycle of *Caulobacter crescentus* can be modeled as a limit cycle oscillator built from two feedback motifs which center on the master regulator CtrA. We found two parameter sets which describe the dynamics: one, which we called ‘weak coupling’, can be parametrized to perfectly describe experimental data in amplitude and phase for DnaA and DivK, with CtrA lagging behind. The second parametrization which we term ‘strong coupling’, removes the constraint put on DivK, and allows a correct fit to DnaA and CtrA. This parametrization requires to reinterpret DivK as its active component, DivK-P. Both model versions differ in their dynamics of cell cycle arrest, where they pass from limit cycle oscillations to the arrest phase via two different types of bifurcations which involve qualitatively different behavior of the limit cycle of the core oscillator, which should be discernible in experiment. Our results suggest that careful experiments trying to resolve the cell cycle conditions approaching arrest for the major regulators can yield interesting insights into the underlying dynamics of the cell cycle and allow to formulate criteria also for the parametrization of more complex models.

ACKNOWLEDGMENTS

Y.V. thanks the Conseil Régional Nord-Pas de Calais for support through a postdoctoral grant via the *Contrat Plan Etat-Région (2007–2013)*.

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