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Control strategies for sustained oscillations in a disrupted biological clock ⋆

Lucie Chambon * **Jean-Luc Gouzé** *

* *Université Côte d'Azur, Inria, INRAE, CNRS, Sorbonne Université, Biocore Team, Sophia Antipolis, France (e-mails: lucie.chambon@inria.fr, jean-luc.gouze@inria.fr).*

Abstract: As many disorders have been correlated with dysfunctional biophysical rhythms, new therapies based on the control of clock functions are emerging for the slowdown of disease progression. In this context, a general disrupted biological clock is modeled by the canonical form of a genetic negative feedback loop. When the unique fixed point of this N-dimensional non-linear differential system is stable, the model reproduces accurately the damped oscillations observed in a damaged oscillator. First, a synthetic modification of the network is proved to generate sustained oscillations and allow to recover a functional clock. The desired periodic trajectories are obtained by destabilizing the fixed point of the model and monotone properties are applied for global results. In a limit case, this modification of the loop is shown to be equivalent to an external piecewise constant control law, supporting the conjecture that simple qualitative control strategies may be able to guarantee sustained oscillations. From the perspective of a biological implementation, this result is promising as these types of control are well adapted to experimental constraints. To support this theoretical work, the methods are applied to the disrupted circadian clock observed in human cancer cells.

Keywords: Feedback loops; Oscillators; Bio control; Nonlinear systems; Qualitative control

1. INTRODUCTION

Gene regulatory networks ensure function, development, and survival of cells in living organisms. Despite their apparent complexity, a small number of reduced and recurrent patterns allow to explain their main functions. Among these building blocks, negative feedback loops are known to be essential for homeostasis (the capacity of an organism to keep an internal parameter constant), and the emergence of sustained oscillations.

Many endogenous biological clocks have been shown to play essential roles, such as the cell cycle and the circadian clock. This latter is essential for an organism to anticipate and adapt its behavior and its physiology to environmental perturbations. Importantly, it has been observed that many diseases such as cancers (Kiessling et al., 2017) or neurodegenerative disorders (Musiek, 2015) are susceptible to cause a disruption of the circadian clock. Alternatively, the synthetic generation of circadian rhythms in disrupted organisms has been proved to be efficient for the slowdown of disease progression (Kiessling et al., 2017). For these reasons, the circadian clock is now considered as a promising tool for therapeutic progress, and especially for cancer treatments. In this context, finding new strategies for the control of biological clocks seems of really high interest.

For this purpose, a large number of biological methods have been developed in the recent years. Besides intrinsic modifications of the genome with engineering tools such as Crispr-Cas9, a lot of external control methods have been created in order to perturb the natural behavior of a genetic network. For example, the introduction of inducer molecules (Lugagne et al., 2017) or the modification of environmental conditions such as the temperature or the osmotic pressure (Uhlendorf et al., 2012) may allow to interfere with the genes and proteins of interest. A more recent technique, called optogenetics, uses modified genes sensitive to specific wavelength of light (Milias-Argeitis et al., 2016) in order to control cells in living tissues. Most of the time, a prior mathematical study has been used in these experiments in order to avoid tedious, repetitive, and onerous biological trials.

Non-linear ordinary differential equations are frequently used for the modeling of gene regulatory networks. In particular, negative feedback loops are conveniently described by "Monotone dynamical systems" as defined in Mallet-Paret and Smith (1990) (see also the Goodwin oscillator in Goodwin et al. (1963)). This class of model has restrictive dynamics, and a lot of results about stability and convergence exist, even in high dimension. Regarding biological control modeling, usual tools and frameworks from classical control theory may not be well adapted due to experimental designs and constraints. Indeed, while classical control strategies require a precise and quantitative knowledge of the system, genetic measurement tools and techniques, such as fluorescent microscopy, can only provide qualitative information of the genetic expression.

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In this context, the resulting differential controlled systems may have discontinuous right-hand sides, and the theory of Filippov (Filippov, 1988) generalizes the definition of solutions.

In this paper, two different control strategies are designed in order to generate sustained oscillations in a disrupted clock that shows arrhythmic behavior. In section 2, the role and the disruptions of the circadian clock are summarized and give a concrete biological motivation for this work. The canonical form of a biological oscillator is modeled in section 3.1 by a non-linear differential system that is also well adapted for the circadian clock (section 3.2). A synthetic modification of the network is shown to generate sustained oscillations in section 4. The local results obtained with the analysis of the corresponding Routh table and the monotone properties of the controlled system allow to show the emergence of global periodic orbits. In section 5, this modified system is proved to converge towards a switching system with discontinuous right-hand side, strengthening the conjecture that periodic orbits may emerge with a simple piecewise constant (PWC) control strategy. To support this hypothesis, this qualitative control method is illustrated in section 6 with the disrupted circadian clock observed in human melanoma cells.

2. A BIOLOGICAL MOTIVATION: THE CIRCADIAN CLOCK

Circadian rhythms are present in many organisms such as plants, molds, insects, and mammals. From the macro-scale (human sleep-wake cycles, body temperature) to the micro-scale (genes, metabolism), these 24-hour self-sustained oscillations are observed everywhere and have been shown to be essential for the anticipation and adaptation to environmental changes. In mammals, natural dark-light cycles generate circadian rhythms in a region of the brain called Suprachiasmatic nucleus, and these oscillations are maintained in the whole organism by a group of genes referred as "clock genes".

It is well known that several diseases, such as sleep disorders (Lippert et al., 2014), cancers (Kiessling et al., 2017) and neurodegenerative diseases (Musiek, 2015), lead to the disruption of circadian oscillations. Conversely, it has been shown more recently that an altered rhythmicity may have several harmful consequences at the metabolic (Staels, 2006) and central nervous system level (Musiek, 2015). These two observations highlight the promising role of circadian rhythms in therapeutic research, especially for cancer treatments. In Kiessling et al. (2017) for example, the authors have developed a new strategy based on the circadian clock to inhibit a tumor growth. Their experiments were based on the observation that clock genes (such as Cry1 or Per2) show arrhythmic expression in human B16 melanoma cells, while they normally exhibit 24-hour oscillations in healthy cells. By enhancing these disrupted genes with different control strategies, such as heat shocks or Dexamethasone introductions, they have been able to restore their rhythmicity, which in turn induced a strong reduction of cancer cells proliferation.

This striking example emphasizes the importance of developing strategies for the control of disrupted biophysical clocks. For this purpose, next section presents a canonical model for biological oscillators.

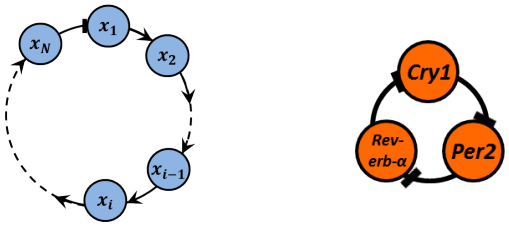

Fig. 1. Left: Directed graph of a canonical negative feedback loop. Right: Directed graph of the reduced mammalian circadian clock model.

3. GENETIC NEGATIVE FEEDBACK LOOP MODEL

It is now well established that genetic negative feedback loops are responsible for biological rhythms and their structural diversity is captured by a standard mathematical model.

3.1 The canonical model

Canonical negative feedback loops in genetics can be modeled by the following non-linear ordinary differential system (see Lugagne et al. (2017) for example):

$$\begin{cases} \dot{x}_1(x_1, x_N) = \kappa_{01} + \kappa_1 \mathrm{h}^-(x_N, \theta_N, n_N) - \gamma_1 x_1, \\ \dot{x}_i(x_i, x_{i-1}) = \kappa_{0i} + \kappa_i \mathrm{h}^+(x_{i-1}, \theta_{i-1}, n_{i-1}) - \gamma_i x_i, \end{cases} \tag{1}$$

$\forall i \in \{2, ..., N\}$. Each variable x_i $\forall i \in \{1, ..., N\}$ represents the concentration of a protein produced with a basal rate $\kappa_{0i} \geq 0$ and degraded with a rate $\gamma_i > 0$. Moreover, $\forall i \in \{2, ..., N\}$ the production of x_i is activated by the protein x_{i-1}, while the production of x_1 is repressed by the protein x_N. The sigmoid Hill functions $\kappa \mathrm{h}^+(x, \theta, n) = \kappa x^n/(\theta^n + x^n)$ and $\kappa \mathrm{h}^-(x, \theta, n) = \kappa(1 - \mathrm{h}^+(x, \theta, n))$ respectively model these two types of interactions with steepness $n \geq 2$, threshold $\theta > 0$, and strength $\kappa > 0$. As biologically required, system (1) is positively invariant.

The structure of these networks is conveniently summarized in a directed graph (see Fig. 1). Importantly, system (1) is considered as the canonical form of negative feedback loops as any loop composed of an odd number of inhibition is fully equivalent to it through a simple change of variable (Mallet-Paret and Smith, 1990). It follows that all the results presented in this paper perfectly apply to this more general class of systems.

It is well known that these negative feedback loops are part of "Monotone dynamical systems" as defined in Mallet-Paret and Smith (1990) for which solutions are restricted to either fixed points or periodic orbits. More precisely, it is possible to show that system (1) has a unique fixed point called $\bar{x}$, and it is numerically observed that either $\bar{x}$ is globally asymptotically stable, or the trajectories converge towards a unique periodic orbit. However, this result has not been shown analytically up to now without restricted hypothesis (see Poignard et al. (2018) for example). From a biological point of view, these dynamics are consistent with the two behaviors that emerge from negative feedback loops in cells, namely homeostasis and sustained oscillations.

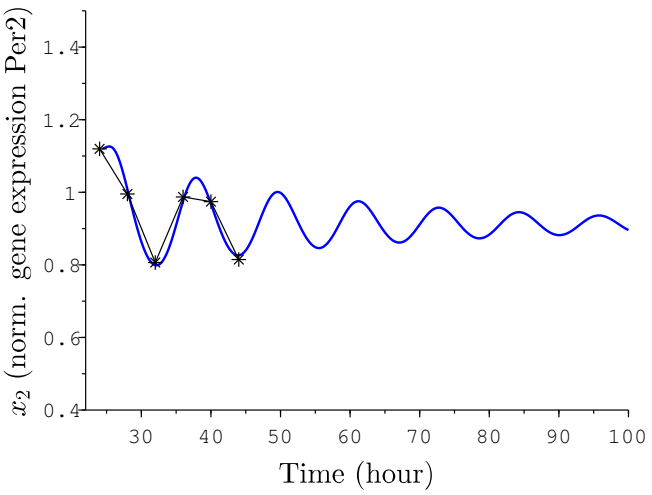

Fig. 2. Calibration of model (2): the black star-plain line is the Per2 arrhythmic data points provided by Kiessling et al. (2017). The blue curve is a simulation of model (2) with $\kappa_{0i} = 0.18$, $\kappa_i = 5$, $\theta_i = 0.38$, $n_i = 4$, and $\gamma_i = 0.36$ $\forall i \in \{1, 2, 3\}$. The initial condition at $t_0 = 24.4$ is $x_0 = (0.81, 1.12, 1.015)$.

In the context of a disrupted clock as explained in the introduction, $\bar{x}$ is considered stable in the rest of the paper such that system (1) generates undesired homeostasis. In sections 4 and 5, two biologically adapted control laws are designed in order to recover a functional biological clock that shows sustained oscillations. These strategies will be illustrated with the circadian clock, for which a reduced model is presented below.

3.2 A reduced circadian clock model

In order to explain and reproduce the oscillatory behavior of circadian clock genes introduced in section 2, quite a few models have been developed. However, the huge number of elements and interactions involved in the network make their mathematical analyses difficult. For this reason, an effort has been made to find reduced models composed of a minimal number of genes and interactions that accurately reproduce the 24-hour oscillations observed biologically. In Pett et al. (2016), a simple negative feedback loop composed of the three clock proteins Cry1, Per2, and Rev-erb-α, is shown to be essential for the emergence of periodic orbits (see Fig. 1). With three inhibitions, this network is equivalent to the canonical structure presented in the previous section and can then be modeled by a generalization of system (1):

$$\dot{x}_i = \kappa_{0i} + \kappa_i \mathrm{h}^-(x_{i-1}, \theta_{i-1}, n_{i-1}) - \gamma_i x_i, \qquad (2)$$

$\forall i \in \{1, 2, 3\}$ where $x_0 = x_3$ and $x_1 = $ Cry1, $x_2 = $ Per2, $x_3 = $ Rev-erb-α.

The parameters of model (2) are calibrated to the Per2 arrhythmic data points measured in B16 melanoma cells during the experiments conducted in Kiessling et al. (2017). For the sake of simplicity, the model is considered symmetric: the parameters κ_{0i}, κ_i, θ_i, n_i, and γ_i are supposed to be equal for any $i \in \{1, 2, 3\}$. The regression is performed by a standard least square routine and the result is shown is Fig. 2: as observed biologically, the oscillations of protein Per2 are damped and converge towards a steady state. This calibration confirms that model (2) is able to capture the dynamics of a disrupted circadian clock.

In section 6, the analytical results will be illustrated with this calibrated model.

4. A SYNTHETIC MODIFICATION OF THE LOOP

In order to obtain sustained oscillations, the desired control law must at least destabilize the fixed point $\bar{x}$. The first selected control leads to the following system:

$$\begin{cases} \dot{x}_1(x_1, x_N) = \kappa_{01} + \mathrm{u}(x)\kappa_1 \mathrm{h}^-(x_N, \theta_N, n_N) - \gamma_1 x_1, \\ \dot{x}_i(x_i, x_{i-1}) = \kappa_{0i} + \kappa_i \mathrm{h}^+(x_{i-1}, \theta_{i-1}, n_{i-1}) - \gamma_i x_i, \end{cases} \qquad (3)$$

$\forall i \in \{2, ..., N\}$, where

$$\mathrm{u}(x) = u_{min} + (u_{max} - u_{min})\mathrm{h}^-(x_N, \omega, m), \qquad (4)$$

and

$$\omega = \left(\frac{1 - u_{min}}{u_{max} - 1}\right)^{1/m} \bar{x}_N, \ u_{min} < 1, \ u_{max} > 1. \qquad (5)$$

For convenience, system (3) will also be noted $\dot{x} = F(\mathrm{u}(x), x)$. For purposes of biological application and in order to facilitate the biological setups, the control law is kept as simple as possible: it only acts on the production of the first protein x_1 and only depends on the concentration of x_N. Moreover, $\mathrm{u}(x)$ stays positive and bounded as biologically required. Finally, this control may be interpreted as a synthetic modification of the network. Indeed, the genetic regulation resulting from the multiplication of $\mathrm{u}(x) = u_{min} + (u_{max} - u_{min})\mathrm{h}^-(x_N, \omega, m)$ with the original decreasing interaction function $\kappa_1 \mathrm{h}^-(x_N, \theta_N, n_N)$ may be produced by multiple and close identical binding sites specific to the transcription factor x_N (Ezer et al., 2014).

For the emergence of oscillations, the steady states of this new synthetic system are identified and analyzed.

Proposition 1. The fixed point $\bar{x}$ of system (1) is also the unique fixed point of system (3) under control law (4).

Indeed, it is easy to see that $\mathrm{u}(\bar{x}) = 1$. Moreover, the monotonic properties of the nullclines allow to prove the uniqueness of the fixed point.

Remark 2. System (3) under control (4) is bounded: $x_1 \in]\kappa_{01}/\gamma_1, (\kappa_{01} + u_{max}\kappa_1)/\gamma_1]$ and $x_i \in [\kappa_{0i}/\gamma_i, (\kappa_{0i} + \kappa_i)/\gamma_i[$ $\forall i \in \{2, ..., N\}$.

The local stability of $\bar{x}$ is investigated with the Jacobian matrix:

Definition 3. The Jacobian matrix of system (1) evaluated on $\bar{x}$ is:

$$J(\bar{x}) = \begin{pmatrix} -\gamma_1 & 0 & \cdots & \cdots & \cdots & 0 & J_1 \\ J_2 & -\gamma_2 & 0 & \cdots & \cdots & \cdots & 0 \\ 0 & J_3 & -\gamma_3 & 0 & \cdots & \cdots & 0 \\ \vdots & \ddots & \ddots & \ddots & \ddots & & \vdots \\ \vdots & & \ddots & \ddots & \ddots & \ddots & \vdots \\ \vdots & & & \ddots & \ddots & \ddots & 0 \\ 0 & \cdots & \cdots & \cdots & 0 & J_N & -\gamma_N \end{pmatrix}$$

where $J_1 = \kappa_1 \mathrm{h}^{-\prime}(\bar{x}_N, \theta_N, n_N) < 0$ and $\forall i \in \{2, ..., N\}$ $J_i = \kappa_i \mathrm{h}^{+\prime}(\bar{x}_{i-1}, \theta_{i-1}, n_{i-1}) > 0$. For system (3) under control (4), the Jacobian matrix $J_u(\bar{x})$ evaluated on $\bar{x}$ is the same as $J(\bar{x})$ where J_1 is replaced by $J_1 + J_{1u}$ with $J_{1u} = \kappa_1 \mathrm{u}'(\bar{x}) \mathrm{h}^{-}(\bar{x}_N, \theta_N, n_N) < 0$.

Proposition 4. The characteristic polynomial associated to $J(\bar{x})$ is:

$$P(X) = \prod_{i=1}^{N}(X + \gamma_i) - \prod_{i=1}^{N} J_i,$$

while the one associated to $J_u(\bar{x})$ is:

$$P_u(X) = P(X) - J_{1u} \prod_{i=2}^{N} J_i, \text{ where } J_{1u} \prod_{i=2}^{N} J_i < 0.$$

When the control parameters u_{min}, u_{max}, and m are fixed, the polynomial $P_u(X)$ is shifted up with respect to $P(X)$. This observation will greatly simplify the determination of the roots of $P_u(X)$: indeed, the addition of the positive term $-J_{1u} \prod_{i=2}^{N} J_i$ to $P(X)$ provokes the propagation of a perturbation in its Routh table, which is investigated in what follows.

From the hypothesis of disrupted biological clock, the fixed point $\bar{x}$ of the uncontrolled system (1) is supposed to be stable as explained in the previous section. As a consequence, $P(X)$ has only roots with negative real part. Moreover, all the coefficients of $P(X)$ are positive, leading to the following proposition:

Proposition 5. All the terms in the first column of the Routh table of $P(X)$ are strictly positive.

From this proposition, a first Lemma can be stated:

Lemma 6. There exists $\tilde{A} > 0$ such that $\forall A > \tilde{A}$, there is at least one sign change in the first column in the Routh table of the polynomial $R(X) = P(X) + A$.

This first lemma easily induces a second lemma:

Lemma 7. There exists $\tilde{m} > 0$ such that $\forall m > \tilde{m}$, the Jacobian $J_u(\bar{x})$ of system (3) under control (4) has at least two complex conjugate eigenvalues with positive real part.

Importantly, this synthetically modified network is part of monotone dynamical systems as defined in Mallet-Paret and Smith (1990), for which strong global dynamical results can be inferred from these two lemmas.

Lemma 8. System (3) under control (4) is an analytic monotone negative cyclic feedback system as defined in Mallet-Paret and Smith (1990).

Proof. With the notations of Mallet-Paret and Smith (1990), system (3) under control (4) can be rewritten:

$$\dot{x}_i = f^i(x_i, x_{i-1}),\ i \in \{1, ..., N\} \tag{6}$$

where $x_0 = x_N$. It is easy to check that:

$$\delta_i \frac{\partial f^i(x_i, x_{i-1})}{\partial x_{i-1}} \geq 0 \quad i \in \{1, ..., N\},$$

with $\delta_1 = -1$, $\delta_i = +1$ otherwise, and the product $\Delta = \delta_1 \delta_2 ... \delta_N$ verifies $\Delta = -1$. Moreover, the functions f^i are only composed of polynomials and rational functions that do not vanish on $\mathbb{R}^+$. It follows that system (3) under control (4) is an analytic monotone negative cyclic feedback system.

Finally, these three Lemmas allow the statement of the main result of this section:

Theorem 9. There exists $\tilde{m} > 0$ such that $\forall m > \tilde{m}$, system (3) under control (4) has one orbitally asymptotically stable non trivial periodic orbit.

Proof. This proof is based on "Theorem 4.3" found in Mallet-Paret and Smith (1990) that can be applied to system (3) under control (4). First, from Lemma 8, this system is an analytic monotone cyclic feedback system with $\Delta = -1$ in ${\mathbb{R}_N}^+$.
Moreover, from Remark 2, this modified system has a compact attractor: $B = \{x_1 \in]\kappa_{01}/\gamma_1, (\kappa_{01} + u_{max}\kappa_1)/\gamma_1]\} \cup \{x_i \in [\kappa_{0i}/\gamma_i, (\kappa_{0i} + \kappa_i)/\gamma_i[\ \forall i \in \{2, ..., N\}\} \subset {\mathbb{R}_N}^+$. From Proposition 1, A contains a single equilibrium $\bar{x}$ and $-\det(-J_u(\bar{x})) = -P_u(0) < 0$ from Proposition 4. Finally, from Lemma 7, there exists $\tilde{m} > 0$ such that $\forall m > \tilde{m}$, the Jacobian $J_u(\bar{x})$ of system (3) under control (4) has at least two complex conjugate eigenvalues with positive real part. Hence, from Theorem 4.3 introduced in Mallet-Paret and Smith (1990), Theorem 9 is proved.

This theorem is convenient as it mainly needs local results on the eigenvalues to deduce global dynamics. The local existence of periodic orbits could have been inferred easily through the emergence of a Hopf bifurcation, but the monotone properties of the modified system greatly improve this result and justify the global emergence of periodic orbits. However, Theorem 9 does not state whether the limit cycle is unique or not. This may be proved with the results presented in Poignard et al. (2018) by replacing Hill functions in system (3) with appropriate saturated functions, and assuming restrictions on the parameters, such as $\gamma_i = \gamma\ \forall i \in \{1, ..., N\}$.

From a biological point of view, this result suggests that an appropriate synthetic modification of the first gene promoter may be a good strategy in order to induce oscillations in a disrupted biological clock. However, due to the tight constraints on the control parameters such as ω, this strategy may be difficult to implement in practice. For this purpose, an extension and a generalization of this result, more adapted for a biological application, is presented in next section.

5. A PWC CONTROL STRATEGY

In order to comply with experimental measurements and inputs constraints, the switching properties of Hill functions are exploited:

Proposition 10. For $m \to +\infty$, control law (4) tends to the following PWC control strategy:

$$\begin{cases} \mathrm{u}(x) = u_{max}\ \forall x_N < \bar{x}_N, \\ \mathrm{u}(x) = u_{min}\ \forall x_N > \bar{x}_N. \end{cases} \tag{7}$$

System (3) under control law (7) is part of differential systems with discontinuous right-hand side for which so-

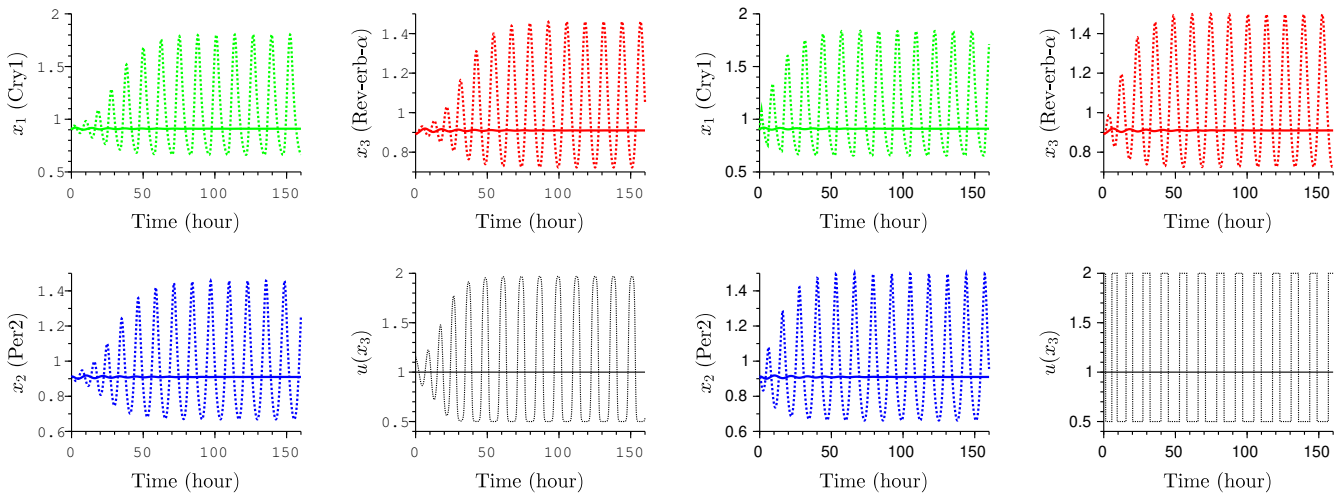

Fig. 3. For all plots, the parameters are the same as in Fig. 2 and the initial condition is $x_0 = (0.9, 0.91, 0.89)$. Without control (depicted by plain lines), the three clock genes $x_1 =$ Cry1 (in green), $x_2 =$ Per2 (in blue), and $x_3 =$ Rev-erb-α (in red) of system (2) show a constant non-cycling expression as expected in the context of a disrupted clock: the system globally converges towards its unique fixed point $\bar{x} = (0.91, 0.91, 0.91)$. With control, the trajectories are depicted by dashed lines. Four left plots: simulation of system (2) under control (4) with $m = 20$, $u_{max} = 2$ and $u_{min} = 0.5$. Four right plots: simulation of system (2) under control (7) with $u_{max} = 2$ and $u_{min} = 0.5$. As expected from Conjecture 11, the three clock genes start to oscillate around their steady state.

lutions are defined in the sense of Filippov as the solutions of the following differential inclusion (Filippov, 1988):

$$\dot{x} \in H(x)$$

such that $H(x) = F(u_{max}, x)$ when $x_N < \bar{x}_N$, $H(x) = F(u_{min}, x)$ when $x_N > \bar{x}_N$, and on the switching domain $x_N = \bar{x}_N$, $H(x) = \bar{co}\{F(u_{min}, x), F(u_{max}, x)\}$, where $\bar{co}$ is the closed convex hull of the set of vector field. The properties of these types of systems are different from classical smooth dynamical systems and must be analyzed carefully with adapted tools and theory.

Theorem 9 proves that the trajectories of system (3) under control (4) oscillate even when the control parameter m is arbitrarily large. Hence, with $m \to +\infty$, it sounds reasonable to infer that control (7) induces oscillations as well, leading to the following conjecture:

Conjecture 11. With $u_{max} > 1$ and $u_{min} < 1$, the trajectories of system (3) under control law (7) converge towards a periodic orbit.

From a biological point of view, this PWC control strategy seems promising and adapted to different biological constraints. Indeed, the measurements of x_N are considered to be of qualitative nature in accordance with partial information provided by biological devices: x_N can either be detected weakly expressed ($x_N \leq \bar{x}_N$) or highly expressed ($x_N \geq \bar{x}_N$). Moreover, the two inputs u_{min} and u_{max} are relevant with the nature of the synthetic control means available in biology that often lead to constant inputs.

6. APPLICATION TO THE CIRCADIAN CLOCK

This PWC strategy is illustrated with the calibrated circadian clock model presented in section 3.2. When m is large enough, the trajectories of system (2) under control (4) (four left plots of Fig. 3) are numerically very similar to the ones emerging under control (7) (four right plots of Fig. 3), corresponding to the following simple control method: if the gene Rev-erb-α is detected highly expressed (resp. weakly expressed), its inhibition on Cry1 must be decreased (resp. increased).

The influence of u_{min} and u_{max} on the characteristics of the oscillations, namely the amplitude and the period, is an interesting open problem. Intuitively, as the parameter u_{max} increases (resp. u_{min} decreases), the x_1-nullcline is shifted up (resp. down): this may induce an increase in the maximum value (resp. a decrease in the minimum value) of the x_1-oscillations, and an increase of the period. This prediction is illustrated in Fig. 4. It is interesting to note that couples of u_{min} and u_{max} can be determined in order to generate 24-hour oscillations, as desired for circadian rhythms.

7. CONCLUSION

In the context of a disrupted biological clock, a synthetic modification of a negative feedback loop has been formulated for the emergence of sustained oscillations. From the local instability of the fixed point, the existence of global limit cycles has been inferred with monotone properties. In a limit case, this synthetic strategy has been shown to be equivalent to a PWC control strategy, resulting in a differential system with discontinuous right-hand side. It has been conjectured that this qualitative method, nicely adapted to biological constraints, is indeed able to generate sustained oscillations in the N-dimensional disrupted negative feedback loop. This method has been numerically illustrated with data providing from melanoma mammalian cells that exhibit arrhythmic clock genes expression.

The control strategies presented in this paper were designed in order to stabilize specifically the unstable fixed point of the differential system, inducing tight constraints

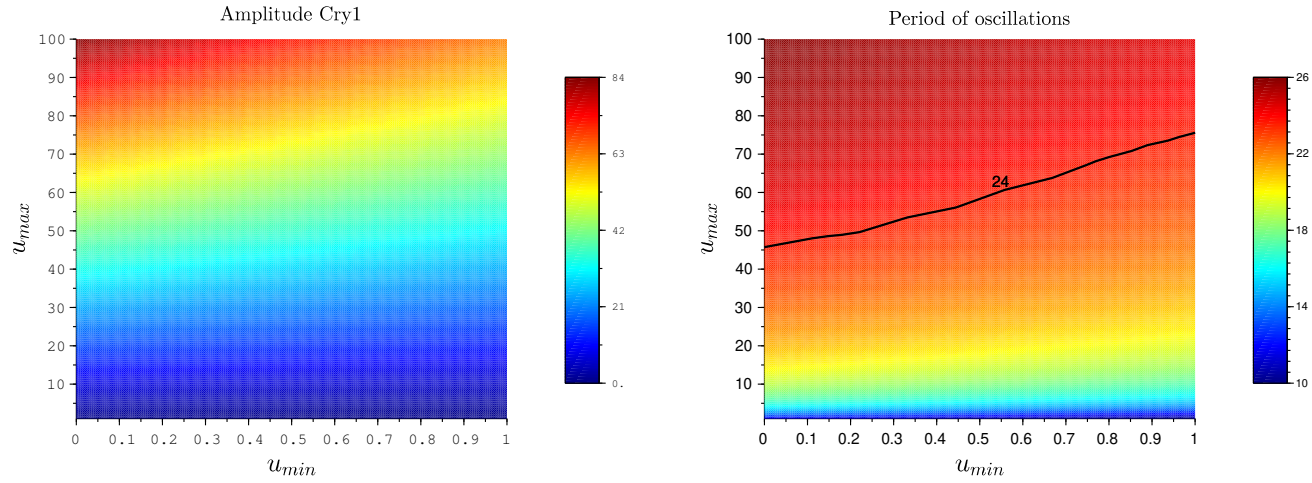

Fig. 4. Influence of the control parameters u_{min} and u_{max} on the amplitude and the period of the oscillations in model (2) with control (7). The parameters are the same as in Fig. 2. Left plot: an increase of u_{max} (resp. u_{min}) increases (resp. decreases) the amplitude of the x_1-oscillations. Right plot: an increase of u_{max} (resp. u_{min}) increases (resp. decreases) the period of the oscillations. The influence of u_{min} seems really limited compared to u_{max} probably due to the fact that u_{max} can be chosen in an unbounded range while $u_{min} \in [0, 1[$. Couples of u_{min} and u_{max} can be obtained such that the period of the oscillations reaches 24 hours (depicted by the dark line).

on the control parameters. It is important to note that these constraints may be relaxed by considering the stabilization of other points, which may not be necessarily the fixed point.

Another extension of this work may be to rigorously show that Theorem 9, proved for the synthetically modified system, applies to the differential system with discontinuous right-hand side. Moreover, it may be interesting to demonstrate that the periodic orbit is unique, and to find an explicit relation between the control parameters and the properties of the orbit, namely its amplitude and its period. Due to the key roles of biological clocks in therapy, it may be really useful to find a simple control strategy capable of independently tuning these two oscillatory properties.

Finally, this study may also apply to different types of biological clocks, such as the cell cycle for example. Just as the circadian clock, this oscillator has been shown to be highly perturbed in the case of various diseases (for example in many cancers) and conversely, its disruption often induces severe damages: its control may be really promising from a therapeutic perspective.

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