Positive Feedbacks Contribute to the Robustness of the Cell Cycle with Respect to Molecular Noise

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Abstract. Most cellular oscillators rely on interlocked positive and negative regulatory feedback loops. While a negative circuit is necessary and sufficient to have limit-cycle oscillations, the role of positive feedbacks is not clear. Here we investigate the possible role of positive feedbacks in the robustness of the oscillations in presence of molecular noise. We performed stochastic simulations of a minimal 3-variable model of the cell cycle. We compare the robustness of the oscillations in the 3-variable model and in a modified model which incorporates a positive feedback loop through an auto-catalytic activation. We find that the model with a positive feedback loop is more robust to molecular noise than the model without the positive feedback loop. This increase of robustness is parameter-independent and can be explained by the attractivity properties of the limit-cycle.

1 Introduction

Biological rhythms occur at various levels of the physiological organisation [16]. They are often generated at the cellular level through complex interactions among genes, proteins, and metabolites [32]. Most cellular

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oscillators rely on interlocked positive and negative regulatory feedback loops [17, 18, 41]. Examples include the so-called Calcium-Induced Calcium Release mechanism responsible for the periodic calcium spiking [31], the p53/Mdm2 oscillator which induces oscillations of p53 in response to stress [6], the Delta/Notch oscillator involved in somitogenesis [34], the CDK/cyclin network controlling the cell cycle [7, 33], and the circadian clock controlling the daily rhythms of the organism [3, 5, 12, 37].

Mathematical modeling of biological oscillators has shown that a single delayed negative feedback loop is sufficient to generate self-sustained oscillations [13, 15, 22, 32]. An experimental demonstration of this prediction was recently brought by synthetic biology [8]: a minimal synthetic oscillator involving genetically engineered gene-promoter constructions was implemented in a bacterium and, in agreement with a theoretical model, exhibits oscillations in gene expression. Thus we may inquire into the role and advantage of additional positive feedback loops observed in most natural cellular oscillators.

Using several prototypical models, Tsai *et al* (2008) [41] performed a series of simulations showing that positive feedbacks lead to a greater tunability of the frequency and to an increase of the domain of conditions (region of the parameter space) which lead to limit-cycle oscillations. Hasty *et al* [25] proposed a theoretical model based on interlocked positive and negative feedback loops and showed that such a design, when coupled to another genetic oscillator, is capable of entrainment and of amplified oscillations. Recently, guided by the predictions of computational models, Stricker *et al* (2008) [40] designed and constructed an artificial oscillator based on interlinked positive and negative feedback loops. This study confirmed that the positive feedback loop enhances the tunability of the system's frequency and increases the robustness of the oscillations over a larger number of conditions.

In the present work, our aim is to check if the positive feedback loop may also lead to a higher robustness of the oscillations with respect to molecular noise. Several works already showed that oscillators based on positive and negative regulatory elements make oscillations more resistant to fluctuations [1, 42], but no comparative study showed how the addition of a positive feedback to an oscillator affects its robustness. We consider here two minimal models for the cell cycle. The first one is only based on a negative feedback. The second one has the same architecture, but incorporates an additional positive feedback. We performed stochastic simulations using the Gillespie algorithm [11] and we quantify the robustness of the oscillations using the auto-correlation function [20] and the distribution of the periods. We show that the positive feedback loop increases the robustness of the oscillations independently of the parameter values, and we provide a possible explanation for this observation.

2 Model

We consider here a minimal model proposed by Goldbeter (1991) for the frog embryonic cell cycle [13]. The model is schematized in Fig. 1 (left panel). The oscillator involves the activation of a cyclin-dependent kinase (CDK1) by Cyclin B, and the CDK1-induced degradation of Cyclin B by an ubiquitin ligase, which is part of the ubiquitin-mediated proteolysis system. Once activated, CDK1 triggers the entry into mitosis.

In an extension of the model, Goldbeter (1993) included an additional positive feedback loop, mediated by the CDC25 phosphatase [14]. In this work, the positive feedback was modeled with an additional variable, standing for the active fraction of CDC25. The latter is activated by CDK1 and, once active, CDC25 activates CDK1. Here we simplify this model by considering a direct feedback of CDK1 on itself (Fig. 1, right panel). This can be seen as an auto-catalytic process.

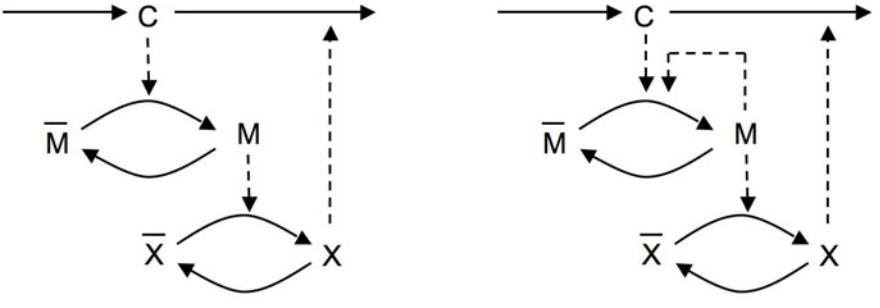

Fig. 1. Schemas of the two models. Left: 3-variable model [13]. Right: 3-variable model including a positive feedback loop (auto-catalysis) (adapted from [14]). Variables C, M, and X denote the Cyclin B, the active form of CDK1 kinase, and the active cyclin protease, respectively. The variables indicated with a bar refer to their inactive form. Solid arrows denote biochemical reactions, while dashed arrows indicate positive regulations.

The time evolution of the three variables is governed by the following system of kinetic equations (see refs. [13, 14] for a detailed description of the equations and the parameters):

$$\frac{dC}{dt} = v_i - v_d X \frac{C}{K_d + C} - k_d C \tag{1}$$

$$\frac{dM}{dt} = v_{m1}(a + bM)\frac{C}{K_c + C}\frac{M_{tot} - M}{K_1 + M_{tot} - M} - v_{m2}\frac{M}{K_2 + M} \tag{2}$$

$$\frac{dX}{dt} = v_{m3} M \frac{X_{tot} - X}{K_3 + X_{tot} - X} - v_{m4}\frac{X}{K_4 + X} \tag{3}$$

Table 1. Stochastic version of the cell cycle model. The variables indicated with a bar refer to their inactive form. Note also that M_{tot} and X_{tot} are here the total number of molecules of M and X. M_{tot} and X_{tot} are obtained by multiplying the concentration as given in the deterministic version by Ω.

No	Reaction	Propensity
1	$\rightarrow C$	$w_1 = v_i \Omega$
2	$C \rightarrow$	$w_2 = v_d X \frac{C}{K_d \Omega + C} + k_d C$
3	$\bar{M} \rightarrow M$	$w_3 = v_{m1}(a\Omega + bM) \frac{C}{K_c \Omega + C} \frac{M_{tot} - M}{K_1 \Omega + M_{tot} - M}$
4	$M \rightarrow \bar{M}$	$w_4 = v_2 \Omega \frac{M}{K_2 \Omega + M}$
5	$\bar{X} \rightarrow X$	$w_5 = v_{m3} M \frac{X_{tot} - X}{K_3 \Omega + X_{tot} - X}$
6	$X \rightarrow \bar{X}$	$w_6 = v_4 \Omega \frac{X}{K_4 \Omega + X}$

In these equations, the variables denote the concentration of Cyclin B (variable C), of active CDK1 kinase (M), and of active cyclin protease (X). Note that in the original version, M and X were the fraction of active CDK and protease but, in order to facilitate the conversion to the stochastic version of the model, we write here all the variables in terms of concentration and consider that the total amount of M and X are M_{tot} and X_{tot}. The term $a + bM$ has been introduced in this version. Parameter a, kept equal to 1 in all the following simulations, controls the negative feedback. The positive feedback is effective when $b > 0$. In the following we will compare the case where $b = 0$ (no positive feedback) and $b = 1$ (effective positive feedback). It is interesting to underly that, in this version of the model, the positive feedback can thus be added continuously through a progressive increase of one parameter (b).

To take into account the fluctuations arising from the limited number of molecules, we need to resort to stochastic simulations. We use here the Gillespie algorithm to simulate a stochastic version of the model as given in Table 1. This Table lists the six reaction steps that define the model as well as their corresponding propensities. These propensities are directly related to the kinetic rates and depend on the number of molecules present in the system, controlled by the system size Ω. Note that we use here directly the Michaelis-Menten functions to compute the propensities. An alternative would be to decompose these kinetics into a set of elementary reaction steps,

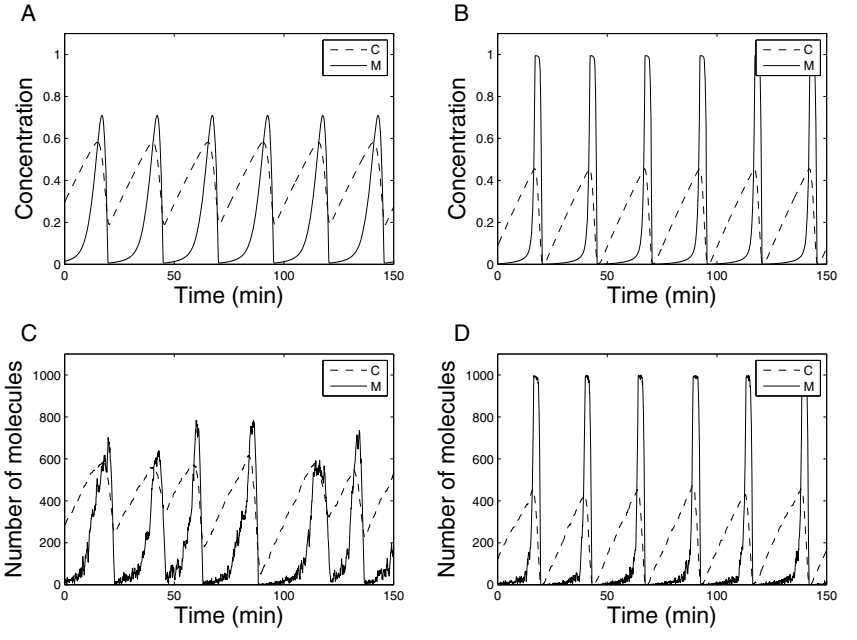

Fig. 2. Deterministic vs stochastic oscillations. (A,C) Model without auto-catalysis ($b = 0$). (B,D) Model with auto-catalysis ($b = 1$). (A,B) Deterministic oscillations. (C,D) Stochastic oscillations for $\Omega = 1000$. Parameter values: $v_i = 0.025$ nM/min, $v_d = 0.25$ nM/min, $K_d = 0.02$ nM, $k_d = 0.01$ min^{-1}, $v_{m1} = 3.0$ min^{-1}, $v_{m2} = 1.5$ min^{-1}, $v_{m3} = 1.0$ min^{-1}, $v_{m4} = 0.5$ min^{-1}, $K_1 = K_2 = K_3 = K_4 = 0.005$ nM, $K_c = 0.5$ nM, $M_{tot} = X_{tot} = 1$ nM, a=1nM. In panels A and B, the concentration is in nM.

but such a decomposition is not straightforward [36] and would lead to a large number of variables and reaction steps, resulting in a level of details unnecessarily high for such a simplified model. Furthermore, theoretical studies have shown that quasi-steady state approximations remain valid in the stochastic case [35].

3 Results

Deterministic simulations of the cascade-based model described above confirmed that oscillations can arise solely as a result of the negative feedback ($b = 0$) [13] (Fig. 2A). The period of the oscillations is around 30 min, which roughly corresponds to the duration of the mitotic cycle in frog embryos, and the shape of the oscillations matches those observed experimentally. Adding a positive loop ($b = 1$) preserves the oscillations but slightly changes their

shape (Fig. 2B). In particular, variables M and X reach a small plateau, near their maximum value M_{tot} and X_{tot}.

Typical stochastic time series obtained by simulating our models with the Gillespie algorithm are shown in figures 2C (for $b = 0$) and 2D (for $b = 1$). For these simulations, we set $\Omega = 1000$, which leads to a number of molecules of few hundreds, a value in agreement with the estimation of the actual number of cell cycle molecules in a cell [27]. In presence of noise, the oscillations still persist but their amplitude and period show some variability. We can already notice that the model with auto-catalysis appears more robust than the model without auto-catalysis.

To understand this increase of robustness in the model with auto-catalysis, it is insightful to examine the dynamics in the phase space. The deterministic and stochastic limit cycles associated to the oscillations shown in figure 2 are given in figure 3 (see the thick close curves in panels A and B). To get a deeper understanding of the dynamics, it is useful to analyze the dynamics (in particular the steady states) of the reduced system obtained when the slow variable C is maintained constant, as described by Tsai et al [41]. In panels A and B, the thin line corresponds to the steady state of M as a function of C. These curves have been obtained by bifurcation analysis of the reduced model defined by eqs. (2) and (3) whith C taken as a parameter. The main difference between the two models is the appearance of a S curve in the reduced model with auto-catalysis. This S curve is associated with bistability. When the dynamics of C is considered (i.e. when the evolution of C is governed by eq. (1)), all variables oscillate, and the 3-ODE system converges to a limit cycle (thick curve on panels A and B). The trajectory follows the upper and lower parts of the S curve and periodically switches from the steady states of the corresponding reduced model (panel B). Two time scales thus appear: a slow motion when the system moves along the upper and lower branches of steady states and a rapid jump from one steady state to the other.

The stochastic trajectories corresponding to figures 3A and B are shown in panels C and D. For the model without auto-catalysis the trajectories are more spread than for the model with auto-catalysis, reflecting the higher robustness of the latter. The dual time scale generated by the positive feedback loop defines regions in the phase space where trajectories are strongly attracted, thereby reducing the spreading of the trajectories of the stochastic system.

To quantify the effect of noise, we computed the auto-correlation function [20] and the period distribution (Fig. 4). Since the entry into mitosis is controlled by the active CDK1, we computed these two measures using variable M. The periods (or, rather, the peak-to-peak intervals) were determined as the time interval separating two successive upward crossings of the mean level of variable M, an arbitrary value which can be seen as the threshold above which mitosis is triggered. We then use the half-life of the decorrelation and the standard deviation of the periods as quantifiers of the

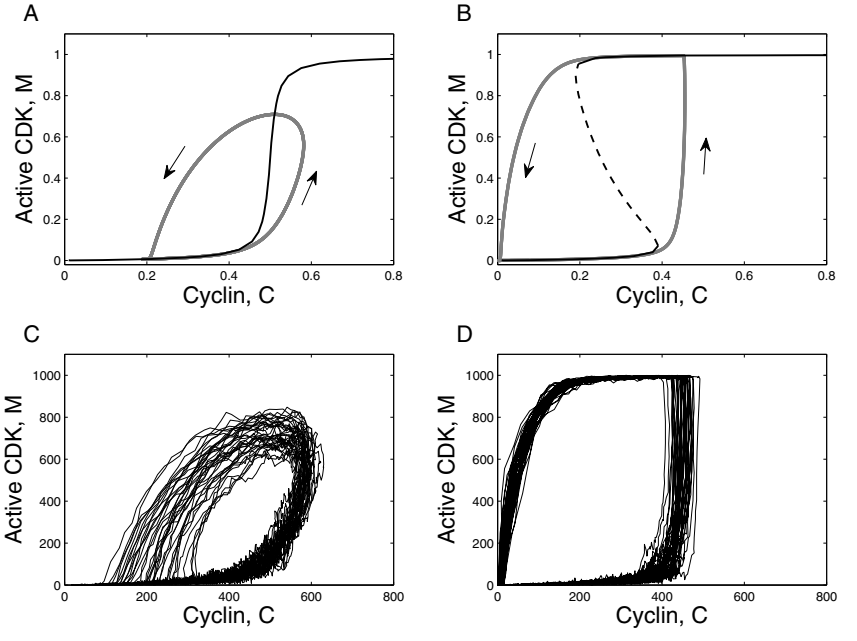

Fig. 3. Deterministic vs stochastic limit cycles. (A,C) Model without auto-catalysis ($b = 0$). (B,D) Model with auto-catalysis ($b = 1$). (A,B) The thin line corresponds to the steady state of M when C is taken as a parameter. The thick grey curve is the deterministic limit cycle of the 3-variable model. The arrows denote the direction along the limit cycle. (C,D) Stochastic trajectories obtained for $\Omega = 1000$. Parameter values are as in Fig. 2. In panel A and B, variables are concentrations (in nM), while in panels C and D, variables are numbers of molecules.

robustness [1, 19, 20]. Comparing the auto-correlation function and the period distribution, it is now obvious that the model with auto-catalysis is more robust than the model without auto-catalysis. Indeed, for the model without auto-catalysis ($b = 0$), the auto-correlation decreases more rapidly and the variability of the period is greater, reflecting a higher sensitivity to noise. Note that the two measures used here rather focus on the robustness of the period of the oscillations. We could have quantified the variation of the amplitude of the oscillations, but from a biological point of view we can hypothesize that mitosis is triggered when a threshold in the concentration of CDK1 is reached and that small variations of the amplitude would not affect the dynamics of cell cycle.

So far we have compared the two models for one parameter set only. However the dynamical properties of the oscillations (amplitude, period, etc) may depend on parameter values. To check if our observations are general, i.e. parameter-independent, we generated for each model about 100 parameter

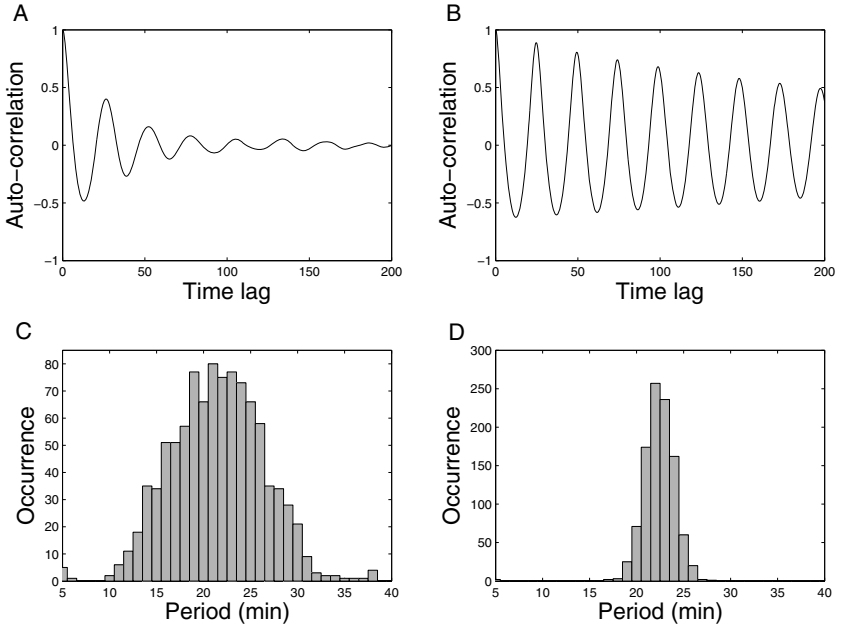

Fig. 4. Quantification of the robustness of the stochastic oscillations (obtained for $\Omega = 1000$). (A,C) Model without auto-catalysis ($b = 0$). (B,D) Model with auto-catalysis ($b = 1$). (A,B) Auto-correlation function. (C,D) Period distribution. These results have been obtained for the time series of M over a time interval of 10000 min. Parameter values are as in Fig. 2.

sets which give limit-cycle oscillations with a period within the range $[30, 40]$ min and a minimal amplitude for M of 0.6. The sets were found using a two-step sampling procedure that yields points uniformly distributed in the volume of the parameter space where these properties are fulfilled [23]. In order to avoid extreme, irrealistic values of some parameters, the sampling is restricted to a region of four orders of magnitude along each parameter, centered on the original parameter set defined in the legend of figure 2. This results in a uniform sampling of the possible parameter sets for which the model matches the predefined criteria. The oscillations were found to be sensitive to parameter a, which controls the negative feedback. In the sampling process we kept $a = 1$ (to maintain the negative feedback) and b was set either to 0(no positive feedback) or to 1 (effective positive feedback).

For both models, we performed stochastic simulations for each parameter set and systematically calculate the half-life of the decorrelation and the standard deviation of the period distribution. The distributions of these two quantifiers are given in Fig. 5. A Wilcoxon rank sum test returned $p - values$

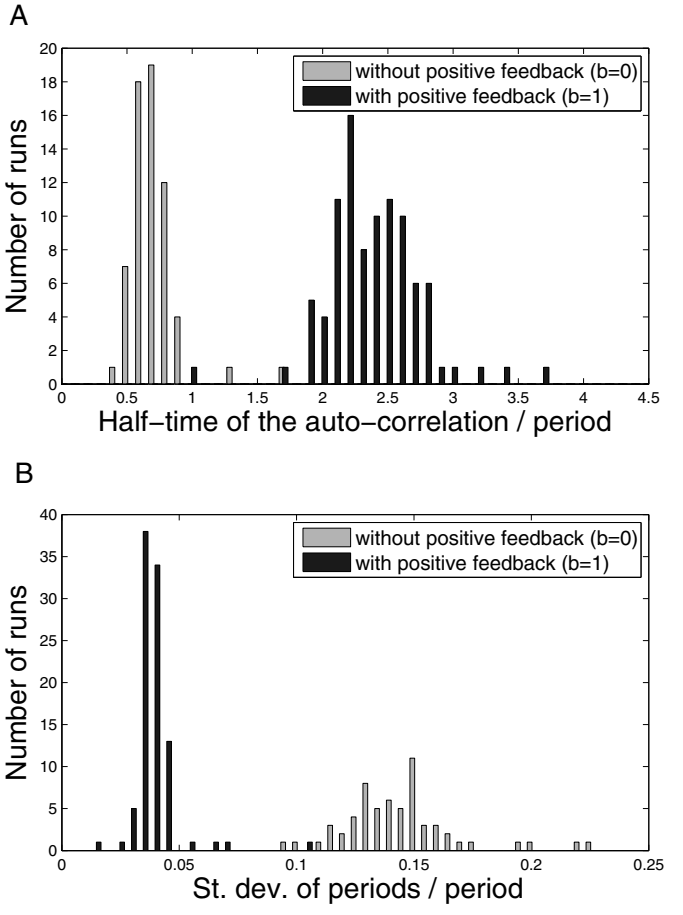

Fig. 5. Robustness of the stochastic oscillations for various parameter sets. (A) Distribution of the auto-correlation half-time and (B) distribution of the periods for the model without ($b = 0$) and with ($b = 1$) the positive feedback loop. For each model about 100 parameter sets have been generated as described in the text. One run has been performed for each parameter set and for each run the time series analysis has been done for variable M over a time interval of 10000 min.

of 2.01E-26 for the auto-correlation and 1.9E-26 for the period distribution, ensuring that we have two distinct distributions. These data thus confirm that the model with auto-catalysis is more robust than the model without auto-catalysis, regardless of parameter values.

4 Discussion

Understanding the design principles of biological oscillations is of general interest in molecular biology [32]. Many cellular oscillators rely on interlinked positive and negative feedback loops. Since a single delayed negative feedback loop has already the potential to generate self-sustained oscillations, the question of the role of additional, positive, feedback loops is open. Besides frequency tunability [41] and oscillations amplification [25], another possible role is illustrated here: positive feedback loops may increase the robustness of oscillators with respect to molecular noise. This role is here highlighted on a minimal three-variable model proposed several years ago for the embryonic mitotic cycle [13]. This model can be seen as a prototypical cascade model and is therefore useful to investigate questions about design. More elaborated models of the cell cycle [10, 26] exist. Stochastic simulations of such a detailed model have recently been carried out by Kar *et al* (2009) [27]. These authors assessed the relative level of intrinsic and extrinsic noise, but they do not address specifically how the oscillator design affects its robustness to molecular noise.

In the future, it would be interesting to perform similar analyses to other simple networks, such as the three-variable Goodwin oscillator, which represents a minimal genetic oscillator, and to extend this work to more detailed models for the cell cycle [10, 26] as well as for circadian clocks [2, 30], which incorporate positive and negative feedback loops.

Robustness to noise is related to regulatory networks topology [1, 4, 28, 38]. Stochastic simulations of minimal models of circadian clocks already put forward several robustness factors that contribute to the robustness of oscillations with respect to molecular noise [9, 19, 21]: cooperativity of gene repression, rate of binding-unbinding of the repressor protein to the gene promoter, forcing by a light-dark cycle, etc. The present study suggests that positive feedback loops, also occurring in circadian clocks [3, 5, 12, 37], may also play a role in the robustness of the oscillations with respect to molecular noise.

Positive feedbacks are typically associated with bistability and hysteresis. They induce several time scales which affect speed and attractivity of the limit cycle and, as already noticed in other works [20, 29, 42], the spreading of stochastic trajectories along the deterministic cycle is correlated with its attractivity properties. This may explain the increase of robustness observed in models based on interlocked positive and negative feedback loops.

Finally, these observations may have implications in understanding evolution of regulatory networks. Indeed, since sensitivity to noise may guide network topology, robustness to noise may be taken as a constraint to design regulatory networks and be added to other constraints such as period and amplitude of oscillations [39, 43]. Interestingly, our model has the property to be able to include or not the positive feedback loop (and to modify its strength) upon tuning of a single parameter and thus opens the possibility

to use robustness to noise as a criterium to study evolution of biological network architectures. As shown in [24], it is possible to evolve in the parameter space continuously while maintaining some macroscopic properties. Our results thus suggest that robustness to noise has contributed to the emergence of additional positive feedback loops and that it may therefore serve as a selection criterion to simulate the evolution of biological oscillators in silico.

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