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ELECTRONIC DESIGN OF SYNTHETIC GENETIC NETWORKS

JAVIER M. BULDÚ
Nonlinear Dynamics and Chaos Group,
Departamento de Física, Universidad Rey Juan Carlos,
Tulipán s/n, 28933 Móstoles, Madrid, Spain

JORDI GARCÍA-OJALVO
Departament de Física i Enginyeria Nuclear,
Univ. Politècnica de Catalunya,
Colom 11, 08222 Terrassa, Spain

ALEXANDRE WAGEMAKERS and MIGUEL A. F. SANJUÁN*
Nonlinear Dynamics and Chaos Group,
Departamento de Física, Universidad Rey Juan Carlos,
Tulipán s/n, 28933 Móstoles, Madrid, Spain
**miguel.sanjuan@urjs.es*

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We propose the use of nonlinear electronic circuits to study synthetic gene regulation networks. Specifically, we have designed two electronic versions of a synthetic genetic clock, known as the "repressilator," making use of appropriate electronic elements linked in the same way as the original biochemical system. We study the effects of coupling in a population of electronic repressilators, with the aim of observing coherent oscillations of the whole population. With these results, we show that this kind of nonlinear circuits can be helpful in the design and understanding of synthetic genetic networks.

Keywords: Synthetic genetic networks; synchronization; nonlinear oscillators.

1. Introduction

Understanding the dynamics of gene regulatory networks is a challenging problem, due to among other reasons the complex connectivity patterns between genes and their regulatory proteins. This complexity has led to the development of synthetic genetic networks [Hasty *et al.*, 2002], much simpler by design than natural ones, and thus easier to understand. These systems are based on interactions between genes that are not linked naturally. Synthetic gene circuits have been designed to reproduce basic functions such as switching [Gardner *et al.*, 2000] or oscillatory dynamics [Elowitz & Leibler, 2000]. These circuits could be combined in order to obtain more complex functions or even interact with natural genetic networks.

In this work, we propose an alternative way of designing and studying synthetic genetic networks, based on the use of nonlinear electronic circuits. Hybrid analog-digital circuits have been already implemented for that purpose [Mason *et al.*, 2004], but in those circuits many features of the original (analog) dynamics are not reproduced faithfully, due to their partially digital character. In this paper we propose to study the dynamics of genetic networks with purely analog

circuits, whose analysis and implementation provides a large flexibility for experimentation. As a case example, we have considered the "repressilator", a genetic network that exhibits oscillations in protein expression [Elowitz & Leibler, 2000]. We propose two different electronic circuits: a first one with operational amplifiers, which reproduces the interactions between genes and proteins in a simple and intuitive way; and a second one with MOS transistors, which would allow integration of a higher number of "electronic repressilators". In both cases we succeed in reproducing the oscillatory behavior reported in the biochemical experiments. Furthermore, we study the effects of coupling in a population of these oscillators, and analyze its influence in the coherence of the global oscillations. The results indicate that coupling is a suitable way of obtaining global oscillations of the whole population. In addition, we show that simple electronic circuits can be useful for the understanding of the dynamics of synthetic genetic networks.

2. The Repressilator

In the repressilator [Elowitz & Leibler, 2000], a chain of three repressor genes, where each repressor inhibits the expression of the following gene, behaves as an oscillator. Figure 1 shows a schematic representation of the interactions inside the network, where arrows represent the promoter sites of the gene. Each repressor gene (in green) produces a protein that binds to the next promoter and stops the production of its corresponding protein. This configuration leads to an oscillation in the expression of the three repressors, with a $2\pi/3$ phase delay.

In its original biochemical design, the period of the oscillations (determined basically by the decay rate of the proteins and mRNA molecules) is larger than the division time of the bacteria that host the network (*E. coli*). After a cell division, the frequencies and phases of the daughter cells drift away from each other, leading to independent oscillations of the whole population. To synchronize the oscillations of the system, inter-cell communication through quorum-sensing has been proposed on the basis of a numerical model [García-Ojalvo *et al.*, 2004].

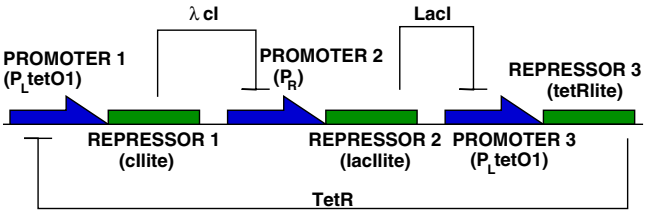

Fig. 1. Network architecture of a synthetic oscillator ("repressilator"). Three repressor genes connected by negative feedback. Promoters (in blue) of each gene (in green) are repressed by proteins (λcI, LacI and TetR) transcribed from the previous gene.

3. The Electronic Repressilator

3.1. *Implementation with operational amplifiers*

Operational amplifiers are common components in a wide variety of electronic circuits [Fiore, 2000]. One of their applications is the construction of modules which compute basic operations such as addition and subtraction. Within this framework we propose an electronic circuit that reproduces the global behavior of the repressilator. The design is shown in the left plot of Fig. 2 and is based on the same principles as the biological repressilator, namely three dynamical elements coupled in chain with an inhibitory interaction.

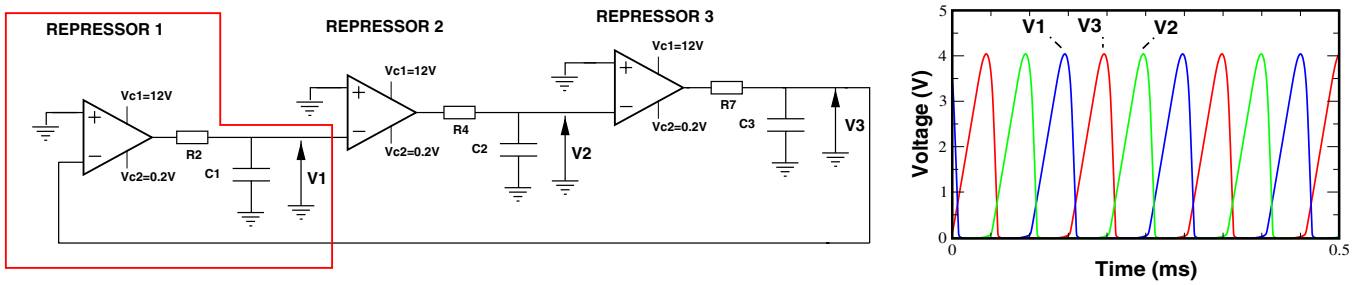

Fig. 2. Electronic setup of the analog repressilator. We used $C_i = 1\mu$ F, $R_i = 1k\Omega$ and UA741 Op-Amps. In the right plot we show the dynamics of the system.

The electronic implementation consists on three basic units made of one RC integrator circuit and one UA741 operational amplifier (OP-Amp). Each voltage measured at the output of the RC circuits (marked as V_i in Fig. 2) is equivalent to the concentration of each repressilator protein. This voltage is further fed into the OP-Amp representing the promoter of the following gene. The output voltage V_i is connected to the negative input and the positive input is set to ground, therefore the OP-Amp is used as a voltage comparator. The output of the amplifier can take only two values: V_{c1} and V_{c2} which are the positive and negative power supply of the OP-Amp. The outputs V_i are linked through the RC circuits in a closed chain, in the same way as the genetic network shown in Fig. 1. The differential equations describing the behavior of the voltage of each unit is expressed by:

$$R_1 C_1 \frac{dV_1}{dt} = -V_1 + H_v(-V_3) \tag{1}$$

$$R_2 C_2 \frac{dV_2}{dt} = -V_2 + H_v(-V_1) \tag{2}$$

$$R_3 C_3 \frac{dV_3}{dt} = -V_3 + H_v(-V_2), \tag{3}$$

where $H_v(x)$ represents the comparator function of the OP-Amp, which can be represented ideally by a step function:

$$\begin{aligned} H_v(x) &= V_{c2} \quad \text{if } x < 0 \\ H_v(x) &= V_{c1} \quad \text{if } x > 0. \end{aligned} \tag{4}$$

The supply voltage is asymmetric, in our case the lower voltage V_{c2} is set to a value slightly below 0 V. In this way the behavior of the circuit is closer to the original genetic oscillatory network and does not display negative voltages. The positive supply is set to $V_{c1} = 12\,\text{V}$. When an output voltage (for example V_2) increases, it induces a reduction of the following output voltage (V_3), since it is injected at the negative input of the corresponding Op-Amp (3 in this case), crossing the threshold $V_{\text{th}} = 0\,V$. Following the chain, V_3, which is decreasing, will enhance the value of V_1 which in turn will decrease V_2. This mechanism leads to an oscillatory behavior (see right plot of Fig. 2) with a frequency and amplitude that depend on the OP-Amp internal parameters but also on the value of R_i and C_i.

Once we have reproduced the dynamics reported in a single unit we are going to analyze the collective behavior of a collection of repressilators. Experimental results *in vivo* have shown that global oscillations are lost due to the out-of-phase dynamics of the system, where each repressilator oscillates with a phase and frequency independent from the others. A quorum sensing mechanism has been proposed as a way to self-synchronize the system [García-Ojalvo *et al.*, 2004]. It consists in inter-cell communication through the exchange of a molecule known as auto-inducer, which allows a global coupling of the system. In the electronic repressilator global coupling can be obtained by taking the voltage of one repressor unit (e.g. V_1), adding the contributions from all units and reinjecting the total signal. Figure 3 shows the temporal evolution of V_1 (corresponding to a protein level) and its corresponding period distribution of a population of $n = 10$ repressilators with global coupling. The internal parameters, which determine the natural frequency of each repressilator, have been adjusted in order to have a distribution of frequencies that varies within 10% around the mean. Coupling is introduced through a resistance R_c placed at V_2 of each circuit and all R_c are connected to a common point. We control the strength of the coupling by adjusting the values of R_c. Note that the coupling strength scales with the inverse of the coupling resistance. The strength of the coupling has been increased from 1% up to 5%, showing that high enough couplings would lead to the synchronization of the whole system, i.e. to the appearance of global oscillations.

3.2. *Implementation with MOSFET transistors*

One of the advantages of using electronic circuits is their integration potential, which allows to construct integrated circuits with a huge number of elements. In this sense, transistors are the most suitable for integration. Since our system consists of a chain of three basic units with negative injection we can replace OP-Amps by transistors keeping the same configuration. Figure 4 (left plot) shows this new setup, where N-channel MOSFET transistors (T1, T2 and T3) act as controllable switches. Protein levels are represented by the output voltages of the transistors (and capacitors).

If the tension applied to the gate exceeds a certain threshold voltage the transistor switches off its output, leading to an output voltage close to zero (the transistor has very low output impedance). In

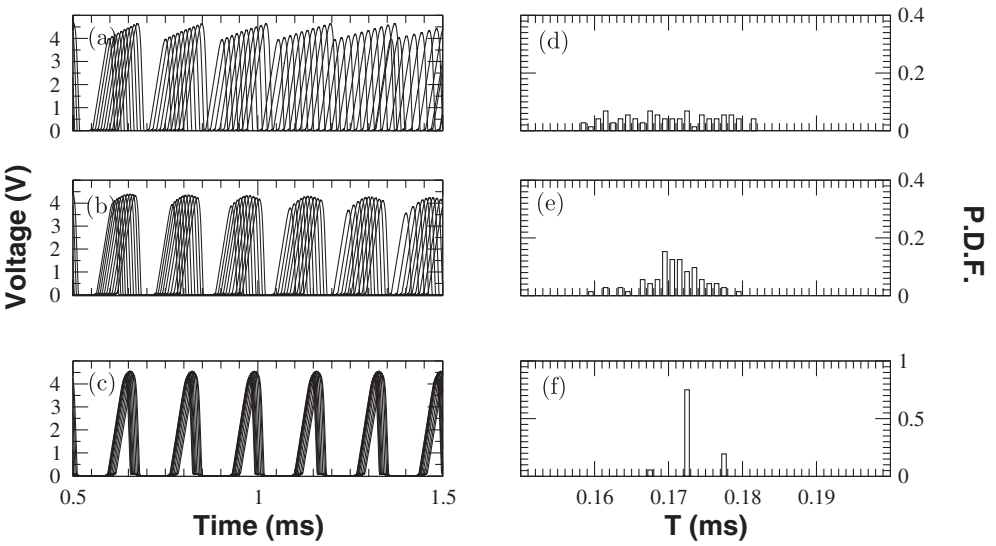

Fig. 3. Time series of V_1 [(a)–(c)] and the corresponding probability distribution function (P.D.F.) [(d)–(f)] of the period of the oscillations for a $n = 10$ repressilator population. We introduce global coupling by reinjecting part of V_1 in a all-to-all configuration. Coupling values are: 0.1% [(a) and (d)], 1% [(b) and (e)] and 5% [(c) and (f)].

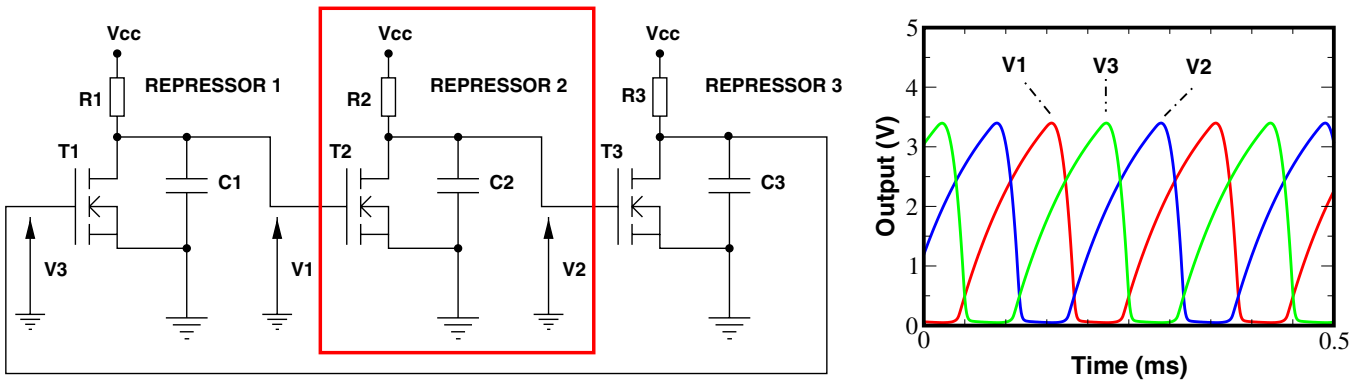

Fig. 4. Electronic setup of an analog repressilator constructed with MOSFET transistors. We used $C_i = 1\mu$ F, $R_i = 1k\Omega$, $V_{cc} = 3.5V$ and 2N7000 MOSFET. The right plot shows the dynamics of this analog repressilator.

this case, the tension on the gate acts as a repressor of the output voltage, similar to what happens with a repressor protein. Let us consider as an example V_2 as the gate voltage of T2 and V_3 as the output voltage. When the gate voltage V_2 falls below threshold (no repression), V_3 is switched on until it reaches its maximum value (the V_{cc} tension) and the transistor acts as a high-level impedance (an open circuit). On the other hand, if repression rises due to an increase of voltage at the previous transistor, the output voltage falls to zero. We can say that the three transistors are repressing themselves in the same way as in the original biochemical repressilator. This kind of configuration leads again to oscillations (see right plot of Fig. 4) at the three output voltages and is known as *ring oscillator*. The differential equations of this system are

$$R_1C_1\frac{dV_1}{dt} = -V_1 + V_{cc}f(V_3) \tag{5}$$

$$R_2C_2\frac{dV_2}{dt} = -V_2 + V_{cc}f(V_1) \tag{6}$$

$$R_3C_3\frac{dV_3}{dt} = -V_3 + V_{cc}f(V_2). \tag{7}$$

The function $f(x)$ depends on the transistor parameters, and should have a sigmoidal shape if we want to obtain oscillations at the transistor's output. A good candidate for $f(x)$ is:

$$f(x) = \frac{\alpha}{1 + \beta x^n}, \tag{8}$$

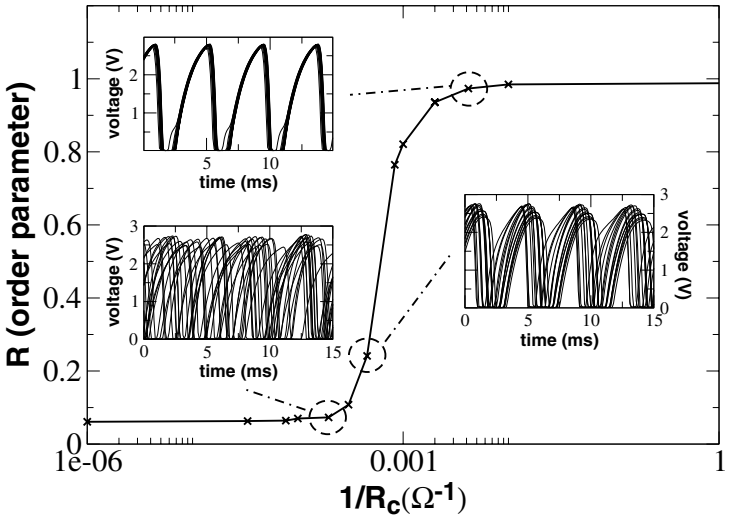

Fig. 5. On the left panel we plot the order parameter R as a function of the global coupling. The insets show the temporal evolution of V_1 for $n = 16$ electronic repressilators. It can be seen that high enough couplings lead to synchronization ($R \sim 1$).

where α, β and n are parameters depending on the MOSFET transistor.

The time evolution of the circuit can be seen in the right plot of Fig. 4. The voltages oscillate with a fixed phase difference of $2\pi/3$ between neighbours. When a transistor (e.g. T1) is active the following one (T2) is repressed and the third (T3) can rise. The repression chain repression is responsible for the oscillations of the whole system, both in electronic and in genetic repressilators.

Now we introduce global coupling into a colony of $n = 16$ repressilators oscillating at different frequencies. Coupling is introduced in the same way as previous section. Figure 5 shows a systematic study of the behavior of the coherence parameter R (see [García-Ojalvo *et al.*, 2004] for details) as a function of coupling. We can observe a phase transition from desynchronized to synchronized behavior at intermediate coupling values.

Acknowledgments

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