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Multistationarity, the basis of cell differentiation and memory.
I. Structural conditions of multistationarity and other nontrivial behavior

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A biological introduction serves to remind us that differentiation is an epigenetic process, that multistationarity can account for epigenetic differences, including those involved in cell differentiation, and that positive feedback circuits are a necessary condition for multistationarity and, by inference, for differentiation. The core of the paper is comprised of a formal description of feedback circuits and unions of disjoint circuits. We introduce the concepts of full-circuit (a circuit or union of disjoint circuits which involves all the variables of the system), and of ambiguous circuit (a circuit whose sign depends on the location in phase space). We describe the partition of phase space (a) according to the signs of the ambiguous circuits, and (b) according to the signs of the eigenvalues or their real part. We introduce a normalization of the system versus one of the circuits; in two variables, this permits an entirely general description in terms of a common diagram in the ''circuit space.'' The paper ends with general statements concerning the requirements for multistationarity, stable periodicity, and deterministic chaos. *© 2001 American Institute of Physics.* [DOI: 10.1063/1.1350439]

In this paper we aim to revisit some classical aspects of nonlinear dynamics under an unconventional light, inspired by the biological background of the authors. More specifically, it emphasizes the key role of positive and negative feedback circuits in biology and nonlinear dynamics. Positive circuits are a prerequisite for multistationarity, whose biological modalities are differentiation and memory. Negative circuits are a prerequisite for stable temporal periodicity and, in biology, homeostasis. Part of this work is a survey of the state of the art with this approach in which we describe earlier results by others and ourselves. The novel part deals with the concept of ''full-circuit,'' i.e., a circuit or union of disjoint circuits that involves all the variables of the system. The notion of full-circuits has been used several times, under different names, to determine nonsingularity criteria for matrices. Here, we show that they play an important role in the dynamics of nonlinear systems.

I. BIOLOGICAL INTRODUCTION

A. Differentiation is an epigenetic process

In multicellular organisms, cell lines differ, not by which genes are present, but by which genes are on and which are off. This was established by the pioneer work of King and Bridges,[1] of Gurdon[2] and of Mintz,[3] and has been recently illustrated in a dramatic way by the cloning of various mammals using nuclei from somatic cells.

In other words, differentiation is an epigenetic process. Epigenetic differences are those which can be transmitted from cell to cell generation in the absence of any genetic difference.

B. Multistationarity can account for epigenetic differences, including those involved in cell differentiation

Multistationarity is the property of systems whose structure is such that they can display two or more distinct (isolated) steady states under identical conditions. That epigenetic processes, including those involved in cell differentiation, can be understood in terms of multiple steady states, has been suggested already long ago by Waddington[4] and most clearly by Delbrück.[5] [Delbrück's historical paper was published in French. An English translation can be found in Thomas and D'Ari.[6] We took the liberty of retranslating ''équilibres de flux'' into ''multiple steady states,'' and were lucky enough to find afterwards the original English manuscript (courtesy of Maurice Fox), in which ''multiple steady states'' is indeed used.]

One of the very first and most beautiful cases of epigenetic differences was discovered and analyzed by Novick and Weiner[7] and by Cohn and Horibata.[8–10] The genes involved in the utilization of the sugar lactose by bacteria such as *Escherichia coli* are expressed only in the presence of a small molecule related to lactose itself and called ''inducer.'' In the presence of moderate concentrations of inducer, the Lac genes in *E. coli* are lastingly on or lastingly off (for 150 cell generations or more) depending on whether or not the culture has been previously exposed for a short period (some minutes) to a high extracellular concentration of inducer. The crucial point is that in this system two cell cultures which are genetically identical, and immersed in identical external conditions, can lastingly display either of two deeply different phenotypes: ''induced,'' if they express the Lac genes,

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''noninduced'' if not. Which phenotype is expressed depends on the previous history, more specifically on the occurrence or not of a brief signal.

The mechanism of this process is well understood; in fact, it had been clearly elucidated already by the early experimentators. The presence of intracellular inducer is required for the synthesis of β-galactoside permease, and in the condition of the experiments (moderate extracellular concentration of inducer) the presence of permease is required for the penetration of the inducer. Thus, in the absence of permease there is a vicious cycle: the inducer does not penetrate for lack of permease, and no permease is synthesized for a lack of intracellular inducer. Clearly, the two epigenetic situations are nothing else than multiple steady states in the parlance of chemists and physicists.

An increasing number of experimental (e.g., Refs. 11 and 12) and theoretical (e.g., Refs. 13–15) works substantiate the notion that multiple steady states can indeed account for epigenetic differences. What is common to all cases is the occurrence of a positive feedback circuit (see Thomas[16] and Thomas and D'Ari[6]).

Circuits will be defined in a rigorous way below (Sec. II). Nevertheless, we describe them already very briefly at this point.

(1) When elements of a system interact with each other in a cyclic way, we say that these elements form a feedback circuit (for short, a circuit).
(2) There are two types of circuits, positive and negative, depending on the parity of the number of negative interactions: if this number is even (including zero), we have a positive circuit, if odd, negative.
(3) The properties of positive and negative circuits are radically different. In short, negative circuits generate homeostasis, while positive circuits are involved in differentiation.

C. Positive feedback circuits are a necessary condition for multistationarity and, by inference, for differentiation

Initially (e.g., Thomas *et al.*[16]), we thought that the occurrence of a positive circuit in a system was merely a simple way to generate multistationarity. It was progressively realized that this statement was too soft and conjectured that a positive circuit is in fact a *necessary condition* for multistationarity. In addition to the many biological examples discovered in the meantime, this conjecture has been subjected to formal proofs.[17–21] In our opinion, however, the domain of validity of these proofs still needs to be extended, because so far they hold only for ''quasi-monotonous'' domains of phase space.

Consider a gene whose product exerts a positive control on its own synthesis. To simplify, we will reason as if the presence of the gene product were both necessary and sufficient for its own synthesis. Clearly, in the absence of the product, the gene will be and indefinitely remain off. In the presence of the product, the gene will be on, and since it is on, more of its product will be synthesized and the gene will remain on.

This one-element positive circuit (''direct autocatalysis'') suffices to account for the fact that a gene subject to positive autocontrol can persist lastingly in either of two stable states, ''on'' or ''off,'' in the same environment. However, in such a case, we have no idea of why the gene is on or off. We do not know either how to switch it from ''on'' to ''off'' or vice versa.

In the biological reality, the decision depends on additional factors. For example, in bacteria carrying bacteriophage lambda, the viral gene (cI), whose product represses all the other genes of the bacteriophage, operates in the presence of its own product or of the product of another gene, cII. The cII product serves to switch gene cI on, and as soon as enough cI product has been synthesized, the gene remains on even though the cI repressor soon switches off gene cII.

In a positive circuit comprising two positive interactions $(+\,+)$, the genes are inter-dependent: they are eventually either both on or both off. Here as well, the absence of the product(s) results in a vicious circle. In contrast, for a positive circuit comprising two negative interactions $(-\,-)$, the genes behave as mutually exclusive: eventually, either gene X is on and gene Y is off, or gene X is off and gene Y is on. If both products are initially absent, either of the two steady states (X on, Y off, or X off, Y on) will take place, depending on such factors as the relative rates of synthesis of the two gene products. Here, in contrast with $(+\,+)$ circuits, no vicious cycle is involved. The intuitive feeling that this situation might result in an alternate periodic expression of the genes is widespread but erroneous, as shown by both the differential and (asynchronous) logical analyses.

Assuming that regulatory interactions are sigmoid in shape, which is very often the case in biological systems, a single positive circuit, whatever its number of elements, can only generate three steady states, two of which are stable. This is because sigmoids compose into steeper sigmoids and not into curves with several inflection points (Reignier *et al.*[22]). In order to account for *many* (hundred or more) cell types in terms of steady states, one needs *several* positive circuits, or else an *ambiguous* circuit (see Sec. II). In fact, m isolated positive circuits can generate up to 3^m steady states, 2^m of which are stable. For example, 8 genes, each located in a positive circuit, can account for up to 256 (2^8) cell types.

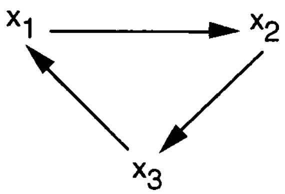
In view of the ubiquity and crucial role of feedback circuits in biological processes, we give here an in-depth analysis of the conditions for multistationarity in terms of circuits. It is followed by a less detailed analysis, again in terms of circuits, of other nontrivial behavior such as stable temporal periodicity and deterministic chaos. The examples given below are abstract ones, which have been chosen to illustrate how simple circuits can generate complex behavior, without any reference to biological application.

II. FORMAL DEFINITION OF FEEDBACK CIRCUITS (FOR SHORT, CIRCUITS)

When a system is described by ordinary differential equations, circuits can be defined in terms of the elements of the Jacobian matrix (see, for example, Eisenfeld and de Lisi[23] and Refs. 17, 21). The idea of describing the interactions in complex systems in terms of the signs of the terms of the Jacobian matrix has been described already long ago by the economists Quirck and Ruppert,[24] without, however, explicit reference to feedback circuits, and by May[25] and Tyson.[26]

Consider the Jacobian matrix of a system of ordinary differential equations. If a term $a_{ij}=(\partial f_i/\partial x_j)$ is nonzero, it means that variations of variable j influence the time derivative of variable i. In this case, we say for short that variable j acts on variable i and we write $x_j \rightarrow x_i$. This action is defined as positive or negative according to the sign of a_{ij}.

Consider now in the Jacobian matrix a sequence of nonzero terms such as a_{13}, a_{21}, a_{32} in which i (row) indices 1, 2, 3 and j (column) indices 3, 1, 2 are circular permutations of each other. Nonzero a_{13} means $x_3 \rightarrow x_1$, nonzero a_{21} means $x_1 \rightarrow x_2$ and nonzero a_{32} means $x_2 \rightarrow x_3$; thus, we have the three-element circuit:



In this way, all the circuits that are present in a system can be read from its Jacobian matrix. More generally, a circuit can be defined as a sequence of terms of the Jacobian matrix such that the sequences of its i (row) and of its j (column) indices are circular permutations of each other.

It is convenient to label circuits as n-circuits according to the number of their elements. As mentioned above, a circuit is positive or negative depending on the parity of the number q of its *negative* interactions; the sign of a circuit is thus defined as $(-1)^q$.

Unions of disjoint circuits are sets of circuits that fail to share any variable. For example, if terms a_{12}, a_{21} and a_{33} are nonzero, we have two disjoint circuits: a 2-circuit between variables x_1 and x_2 and a 1-circuit involving variable x_3.

Eisenfeld and de Lisi[23] have already introduced a generalization of the concept of circuit, for which they have coined the term ''*generalized cycle*'' (''*g*-cycle''): ''a *g*-cycle is a set of vertex disjoint cycles, i.e., any two cycles do not share a common vertex.'' They also introduced the important concept of the ''sign of a *g*-cycle'' defined as $(-1)^{(p+1)}$, in which p is the number of positive cycles involved. In other words, a *g*-cycle is positive if it comprises an *odd* number of *positive cycles*. One can check that this definition of the sign of a *g*-cycle includes the particular case of a simple cycle.

We prefer to use ''circuit'' rather than ''cycle'' (or ''loop'') in order to remain consistent with the nomenclature used in graph theory. In addition, we are often lead to treat the sign of simple circuits and of unions of two or more disjoint circuits separately, because the sign of a circuit has a simple and obvious functional meaning, while it is not the case for the sign of a union. Yet, a general and elegant definition suggested by Cahen[27] applies both to circuits and unions of disjoint circuits. They can be identified by the existence of a set of nonzero terms of the matrix, such that the sets of their i (row) and j (column) indices are equal.

Circuits as well as unions of disjoint circuits can be described in three equivalent ways: as a graph of interactions, as sets of elements of a Jacobian matrix or as the product of relevant terms of this matrix.

We wish to emphasize that whenever one deals with a nonlinear system one or more terms of the Jacobian matrix is a function of variable(s) of the system. It ensues that *a given circuit can have a different sign depending on the location in phase space.* Such ''ambiguous'' circuits occur very often and when this is the case, phase space can be partitioned into domains within each of which the signs of the circuits are constant (see Sec. III B).

From the Jacobian matrix, one derives the characteristic equation, whose solutions are the eigenvalues of the matrix. In what follows, however, rather than calculating the values of the eigenvalues near the steady states only, we will keep their analytical expression, and consider them not only at the level of steady states but everywhere in phase space. Phase space can then be partitioned into domains according to the signs of the eigenvalues or their real part, and the nature of any steady state will depend on the domain within which it is located. It was found informative to compare this partition with that based on the signs of the circuits.

The prominent role played by the circuits is no more surprising once it is recognized that among the terms of the Jacobian matrix, only those belonging to a circuit are present in the characteristic equation. Consequently, only those terms of the Jacobian matrix that belong to a circuit contribute directly to the eigenvalues. The off-circuit terms of the Jacobian matrix provide a one-directional connection between otherwise disjoint circuits; as a result, they may influence the steady state values of the variables. Although they are not present in the Jacobian matrix, constant terms in the ODE's also influence the location of the steady states. Thus, both off-circuit terms of the Jacobian matrix and constant terms in the ODE's play a role in the system's dynamics. However, their role is only indirect via their effect on the location of the steady states.

III. FULL-CIRCUITS

A. Definition of full-circuits

Those circuits and unions of disjoint circuits that involve all the variables of a system play a special role in the relation between circuits and steady states. This concept is not new. It appears in the literature under different names, e.g., ''*g*-cycles of length *n*,''[23] ''diagonal products''[28] or ''transversal products.''[29] We call them ''*full-circuits*,'' irrespective of whether they are circuits or unions of two or more disjoint circuits. Figure 1 shows three of them in the case of three-variable systems. More generally, there is a very simple algorithm for extracting the complete list of the full-circuits from the Jacobian matrix. It simply consists of writing the analytic form of the determinant of the matrix. In an n-variable system, this determinant is a sum of n-factor products and each product corresponds to one of the ''full-circuits'' of the system. For example, for a three-variable system, the determinant of the Jacobian matrix is

$$\begin{bmatrix} \cdot & a_{12} & \cdot \\ \cdot & \cdot & a_{23} \\ a_{31} & \cdot & \cdot \end{bmatrix} \qquad \begin{bmatrix} \cdot & a_{12} & \cdot \\ a_{21} & \cdot & \cdot \\ \cdot & \cdot & a_{33} \end{bmatrix} \qquad \begin{bmatrix} a_{11} & \cdot & \cdot \\ \cdot & a_{22} & \cdot \\ \cdot & \cdot & a_{33} \end{bmatrix}$$

FIG. 1. Three examples of ‘‘full-circuits’’ (circuits and unions of disjoint circuits which involve all the variables) in three-variable systems.

$$a_{11}.a_{22}.a_{33}-a_{11}.a_{23}a_{32}-a_{22}.a_{31}a_{13}-a_{33}.a_{12}a_{21}$$
$$+a_{12}a_{23}a_{31}+a_{13}a_{32}a_{21}.$$

The six terms correspond to the six full-circuits that can exist in a three-variable system. Note that in the expression of the determinant, they are affected by a ‘‘signature’’ (+ or −), but this point will not have to be taken into account here.

Consider a system made of a single full-circuit without any additional terms (a ‘‘pure’’ full-circuit). In such a system, the nature of the eigenvalues (their signs, real or complex character) is entirely determined by the signs of the circuits. This results from the following.

(1) For an isolated circuit of n elements, whatever its detailed structure (nonlinearities, parameter values, signs of the individual elements, etc.,...), the n real or complex eigenvalues are nothing else than the n values of $\lambda = p^{1/n}$, in which p is the product of the relevant terms of the Jacobian matrix. In particular, the eigenvalue corresponding to a one-element circuit (diagonal term of the Jacobian matrix) is identical with this term itself.

(2) If the pure full-circuit is a union of disjoint circuits, these disjoint circuits can be treated independently of each other, and the eigenvalues of a system made of disjoint circuits is the union of the eigenvalues corresponding to these disjoint circuits.

Consequently, a pure (and nonambiguous) full-circuit can generate only a well-defined *type* of steady state: the signs and real vs complex nature of the eigenvalues are entirely determined by the signs of the component circuits. For this reason, it is justified to denote this type of steady state as characteristic of the full-circuit in question. In contrast, an ambiguous full-circuit can generate (and be characteristic of) two or more types of steady states, as we will see in Sec. III B.

Table I gives, for 2-variable systems, all the combinations of signs in nonambiguous pure full-circuits, and in each case the nature of the corresponding eigenvalues and of the type of steady state. For 3-variable systems, we give only some examples. As can be shown analytically, systems comprising only a positive 3-circuit have a steady state (a saddle-focus) with eigenvalues of the type (+/−−), that is, there is one real positive root and a pair of conjugate complex roots with a negative real part. To illustrate this point, consider the system:

$$\dot{x}=y, \quad \dot{y}=z, \quad \dot{z}=x^3,$$

whose Jacobian matrix is

$$\begin{pmatrix} 0 & 1 & 0 \\ 0 & 0 & 1 \\ 3x^2 & 0 & 0 \end{pmatrix}.$$

In view of the choice of the nonlinearity, the 3-circuit is necessarily positive ($3x^2$ has a constant sign) and there is, as just mentioned, a steady state of type (+/−−)

Similarly, a system composed of a negative 3-circuit has a steady state with eigenvalues of type (−/++). A system which consists of a two-element positive circuit (++ or −−) and a one-element negative circuit on the third variable has a steady state with eigenvalues of type (+−−). A system with only diagonal terms has eigenvalues equal to these diagonal terms.

B. Ambiguous full-circuits

A circuit can be positive or negative depending on the location in phase space, and we then call it *ambiguous*. A full-circuit is ambiguous if one or more of its component circuits is itself ambiguous. Whenever a full-circuit is ambiguous, it can generate two or more types of steady states, depending on the location in phase space. The example given below has intentionally been chosen as simple as possible:

$$\dot{x}=y, \quad \dot{y}=x^2-1,$$

whose Jacobian matrix is

$$\begin{pmatrix} 0 & 1 \\ 2x & 0 \end{pmatrix}.$$

There is a single, two-element full-circuit. As regards the sign of this circuit, phase space is cut into two regions: the circuit is positive for $x>0$, negative for $x<0$. There are two steady states (−1,0) and (+1,0), one in each region, and, as

TABLE I. Strictly speaking, this table is valid only for pure full-circuits. To what extent it also holds for nonzero values of the other elements of the Jacobian matrix, is described in Sec. IV B.

Pure full-circuits in 2 variables		
The 2-circuit	Eigenvalues	Steady state
Positive $\begin{pmatrix} & - \\ - & \end{pmatrix}$ or $\begin{pmatrix} & + \\ + & \end{pmatrix}$	(+,−)	Saddle point
Negative $\begin{pmatrix} & + \\ - & \end{pmatrix}$ or $\begin{pmatrix} & - \\ + & \end{pmatrix}$	complex	Center[a]
The unions of two 1-circuits		
$\begin{pmatrix} - & \\ & - \end{pmatrix}$	(−,−)	Stable ‘‘node’’
$\begin{pmatrix} - & \\ & + \end{pmatrix}$ or $\begin{pmatrix} + & \\ & - \end{pmatrix}$	(+,−)	‘‘Saddle point’’
$\begin{pmatrix} + & \\ & + \end{pmatrix}$	(+,+)	Unstable ‘‘node’’

[a] If the diagonal elements are nonzero, the steady state is a focus, stable or unstable depending on the sign of the trace.

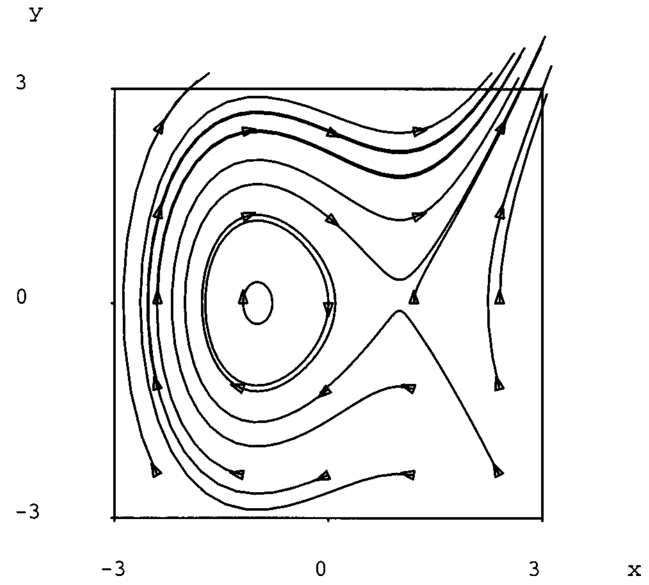


FIG. 2. $\dot{x}=y, \dot{y}=x^2-1$, whose Jacobian matrix is $\left(\begin{smallmatrix}0 & +1\\ 2x & 0\end{smallmatrix}\right)$. An ambiguous full-circuit. Phase space is partitioned by $x=0$ into two domains. For $x>0$ the circuit is positive, for $x<0$, the circuit is negative. There are two steady states, a saddle point in the positive domain, a center in the negative domain.

expected from the sign of the circuit, the first steady state is a saddle point and the second steady state is a center (see Fig. 2).

C. A tight relation between full-circuits and steady states

1. Absence of any full-circuit

As mentioned above, the full-circuits of a system are the nonzero terms of the analytic expression of the determinant of the Jacobian matrix. If there are no full-circuits, this determinant is thus zero everywhere in phase space. In this condition (in which the Jacobian matrix is singular and one or more eigenvalue is zero), the system of steady state equations is under-determinate. This has in fact been shown already by others (see e.g., Refs. 23, 25 and 28) using another terminology.

2. Multistationarity requires the presence, either of two full-circuits of opposite signs, or of an ambiguous full-circuit

We realized long ago that the presence of a positive circuit in the Jacobian matrix of a system is a necessary, but not sufficient, condition for multistationarity. The presence of a positive circuit can generate two basins delimited by a separatrix, and this even in a linear system. However, in order actually to have multistationarity, the presence of an appropriate nonlinearity is of course required, if only for trivial algebraic reasons. In addition, when there is more than one steady state, invariably there is more than one *type* of steady state. In view of the tight relationship between the nature of steady states and the structure of full-circuits, one would thus expect multistationarity to require at least two types of full-circuits, or else an ambiguous full-circuit.

This concept of two ‘‘types’’ of full-circuits remained somewhat unclear until we realized that the two ‘‘types’’ might be nothing else than two full-circuits of opposite signs according to the definition of Eisenfeld and de Lisi given in Sec. II. The conjecture thus becomes ‘‘*In order to have more than one steady state, one needs an ambiguous full-circuit, or two full-circuits of opposite signs.*’’

This statement encompasses our older statement that the presence of a positive circuit (of any length) somewhere in phase space is a necessary condition for multistationarity. Indeed, a full-circuit that is positive according to the definition of Eisenfeld comprises necessarily a positive (simple) circuit. Thus, the presence of a full-circuit of each sign does imply the presence of a simple positive circuit.

In contrast, the presence of a negative full-circuit does not imply the presence of a negative circuit: a negative full-circuit comprises an even number of positive circuits, independently of the number of negative circuits, which may be zero. That multistationarity does not require the presence of any negative circuit, is demonstrated by a simple system, which comprises two nonambiguous full-circuits, one positive, made of a positive 2-circuit, one negative, made of two positive 1-circuits (although there are only positive circuits, the two full-circuits are of opposite signs according to the definition of Eisenfeld):

$$\dot{x}=x+y, \quad \dot{y}=x^3+y,$$

whose Jacobian matrix is

$$\begin{pmatrix} +1 & +1 \\ 3x^2 & +1 \end{pmatrix}.$$

(To show that process *b* is a necessary condition for process *a*, one needs a formal demonstration. To show that process *b* is *not* a necessary condition for process *a*, a single example of the occurrence of *a* in the absence of *b* is sufficient.)

This system has three steady states, $(-1,1)$, $(0,0)$ and $(1,-1)$, two saddle points and an unstable node. The reason for using x^3 as the nonlinearity of the system is again that its derivative, present in the Jacobian matrix, has a constant sign $(+)$; in other words, there is no ambiguity. If the nonlinearity had been rather x^2, a single full-circuit would have been sufficient to generate multistationarity (see Sec. III B).

We mentioned above that the conjecture concerning the requirement for two full-circuits of opposite signs has no formal proof yet but strong circumstantial evidence. In addition to the occurrence of a number of favorable cases and to the absence, so far, of any counter-example, we can add at the present time the following argument. We have constructed the complete list of the 2*2 and 3*3 matrices with 0, $+1$ and -1's. There are 81 (3^4) matrices in the first case and 19683 (3^9) in the second case. From these lists we have selected the matrices that contain no full-circuit, those with a single full-circuit, with two or more full-circuits of the same sign, with two or more full-circuits of different signs, etc. From these matrices, we have constructed differential equations with random coefficients and independent terms and a nonlinearity (x^3) such that the circuits cannot be ambiguous.

It was found that in every case tested (all for 2 variables, several arrays of 100 for 3 variables), the systems of equations created from the matrices without a full-circuit led to under-determinacy, in agreement with statement (1). Those from matrices with a single nonambiguous full-circuit or with two nonambiguous full-circuits of the same signs, yielded a single real root for any choice of parameters in the differential equations. As for the equations from matrices with two nonambiguous full-circuits of different signs, they have three solutions, in agreement with the nature (x^3) of the nonlinearity chosen. Depending on the case, one finds three real solutions (and thus multistationarity) or one real and two complex solutions. In the latter case, we repeated the run with other choices of the random coefficients and independent terms; each time this was done, cases with three real roots were found after some runs. This strongly suggests that, provided the above-mentioned conditions on the full-circuits are fulfilled, one can find simple nonlinearities and parameter values such that the system displays multistationarity.

3. Multistationarity generated by a single, ambiguous full-circuit

A system that comprises a single but ambiguous full-circuit may display multistationarity for proper values of the parameters and an appropriate nonlinearity, as illustrated in the section on ambiguous circuits.

4. Location of the nonlinearity

The nonlinearity in the differential equations and the corresponding variable term(s) in the Jacobian matrix do not have to be located on the positive circuit required for generating multistationarity. However, as far as we can tell from many examples, it has to be located on a full-circuit. In the system

$$\dot{x}=x-y^3, \quad \dot{y}=x-y,$$

with the Jacobian matrix

$$\begin{pmatrix} +1 & -3y^2 \\ 1 & -1 \end{pmatrix},$$

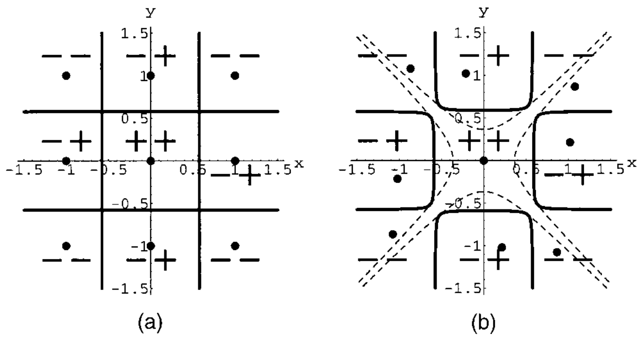
the only positive circuit is in a_{11} but the nonlinearity is on the 2-circuit.

IV. SYSTEMS COMPRISING A FULL-CIRCUIT AND ADDITIONAL NONZERO ELEMENTS IN THE JACOBIAN MATRIX

It has been stressed above that in the case of a pure full-circuit the nature of the steady state(s) can be inferred by simple inspection of the analytic expression of the Jacobian matrix. The situation is of course less simple if other elements of the Jacobian matrix are nonzero in addition to the full-circuit considered. We will now do the following.

(i) Describe simple examples of two-variable systems comprising the two full-circuits and nonlinearities on the diagonal or on the nondiagonal elements.

(ii) Develop a normalization that permits one to describe any two-variable system, whatever the parameter values and the number and location of the nonlinearities, in terms of a common diagram in the ‘‘circuit space’’ and briefly allude to three-variable systems.



(a) (b)

FIG. 3. $\dot{x}=x-x^3+cy, \dot{y}=dx+y-y^3$, whose Jacobian matrix is $\begin{pmatrix}1-3x^2 & c \\ d & 1-3y^2\end{pmatrix}$, (a) with $c=d=0$; (b) with $c=0.2$ and $d=-0.2$. The partition according to the signs of the 1-circuits (whatever the values of c and d) is shown in (a). The partition according to the signs of the eigenvalues or their real part is shown, again by (a) for $c=d=0$, and by (b) for $c=0.2$ and $d=-0.2$. The eigenvalues are complex inside the dotted curves. The 9 steady states are represented by points.

A. Two-variable systems

Consider the system

$$\dot{x}=x-x^3+cy, \quad \dot{y}=dx+y-y^3, \quad \text{with } c,d \in R,$$

whose Jacobian matrix is

$$\begin{pmatrix} 1-3x^2 & c \\ d & 1-3y^2 \end{pmatrix}.$$

Each of the two terms of the form $(1-3u^2)$ is positive for $|u|<1/\sqrt{3}$ and negative outside. Thus, phase space is cut into 9 (3^2) boxes in which the 1-circuits are alternatively positive and negative. Let us first consider the extreme case in which the nondiagonal elements are absent. As in this case one deals with a pure full-circuit, the partition of phase space according to the signs of the eigenvalues or their real part coincides with the partition according to the signs of the circuits. These signs can be directly translated into the signs of the eigenvalues according to Table I. There are in fact 9 steady states of the expected nature, one per box [Fig. 3(a)].

We are of course more interested in the general case, in which the nondiagonal terms have to be considered. Figure 3(b) shows the partition of phase space according to the signs of the eigenvalues for $c=0.2$ and $d=-0.2$. It can be seen that the partition strongly resembles that found in the absence of the nondiagonal terms, and, hence, to the partition based on the signs of the circuits. Phase space is still divided into 9 basins, each of which contains a steady state of the expected nature. Figure 3(b) shows, in addition, the curves inside which the eigenvalues are complex. As the absolute values of parameters c and d still increase, the situation progressively departs from the initial one and steady states are lost.

If the nonlinear terms had been placed instead at the nondiagonal positions, the partition lines would have been the same but the signs of the eigenvalues would have been

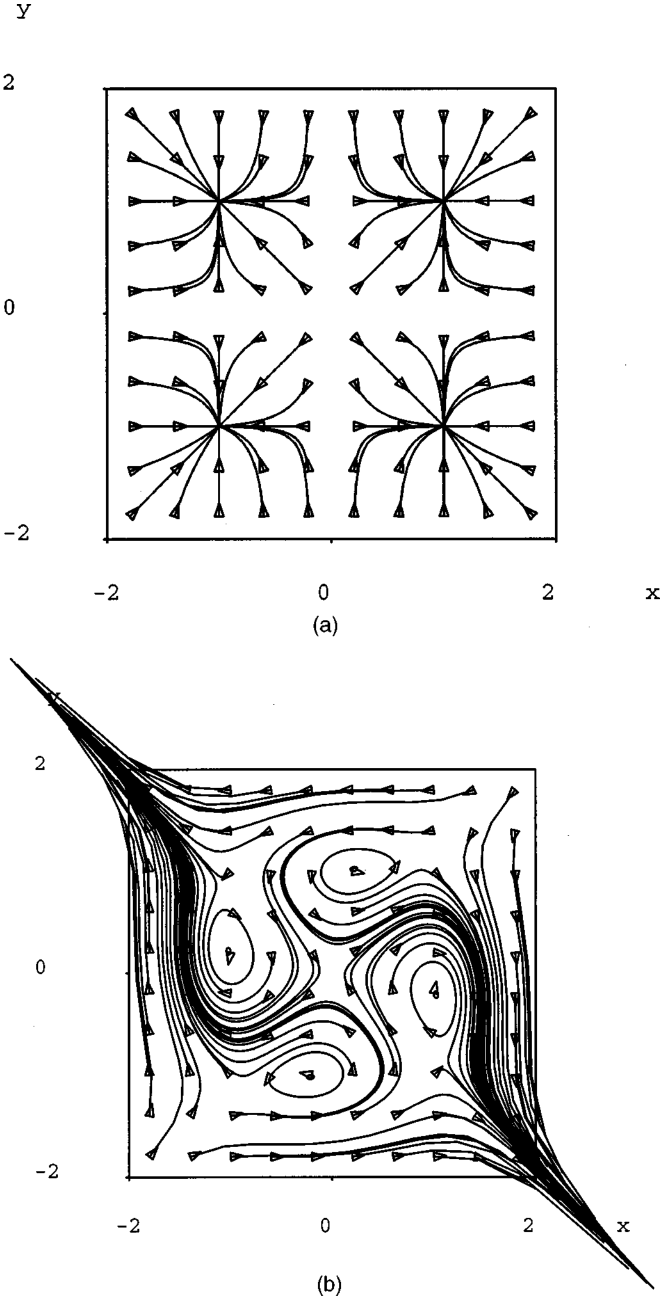


FIG. 4. Trajectories of the systems, (a) $\dot{x}=x-x^3+0.2y, \dot{y}=-0.2x+y-y^3$, whose Jacobian matrix is $\begin{pmatrix}1-3x^2 & +0.2\\ -0.2 & 1-3y^2\end{pmatrix}$; (b) $\dot{x}=0.2x+y-y^3, \dot{y}=x-x^3-0.2y$, whose Jacobian matrix is $\begin{pmatrix}+0.2 & 1-3y^2\\ 1-3x^2 & -0.2\end{pmatrix}$. In both cases, the partition follows the unbroken lines of Fig. 3(b). However, in the second case the 9 steady states are an unstable node, four saddle points and four centers.

different (and just as easily predictable). Figures 4(a) and 4(b) shows trajectories for the two systems (both with $c=0.2$ and $d=-0.2$).

B. Normalization

In n variables, we have up to n^2 terms a_{ij} corresponding to nonzero elements of the Jacobian matrix. As these terms form products that represent circuits, one can reduce their number and simplify the description by applying a normalization according to an appropriate circuit. Normalizing according to an n-circuit represented by the product c consists of dividing all the terms of the Jacobian matrix by the positive real $|c|^{(1/n)}$. At the level of the eigenvalues, this simply results in dividing them by the positive real number in question, without affecting their signs. This approach is a reformulation of the standard classification of the dynamics. Its interest lies in classifying the dynamic properties in terms of the sign and relative weight of the circuits. For the sake of simplicity, we describe the approach in 2 variables, although it is only for higher dimensionalities that it will become really useful.

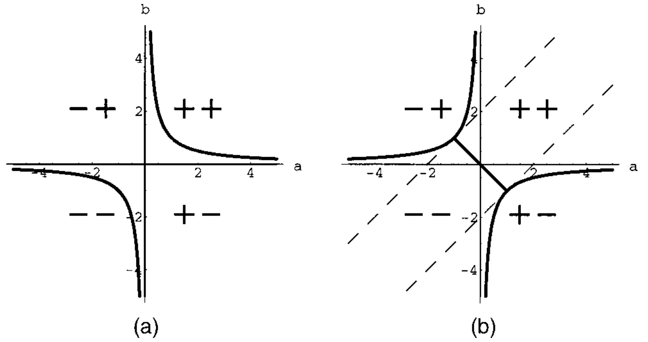


FIG. 5. Normalization of 2-variable systems (for the 2-circuit). (a) The 2-circuit is positive. (b) The 2-circuit is negative. The signs of the eigenvalues correspond to the signs of the quadrants, except in the crescents between the axes and the hyperbola (in these regions, $|a\ b|<1$, which means that the diagonal full-circuit is ''dominated'' by the nondiagonal full-circuit). In (b), the eigenvalues are complex between the two dotted lines.

In 2-variable systems, one can normalize by the circuit $a_{12}a_{21}$ by dividing all four terms of the Jacobian matrix by $|a_{12}a_{21}|^{(1/2)}$ provided, of course, neither a_{12} nor a_{21} is zero. The diagonal terms are thus replaced by $a=a_{11}/|a_{12}a_{21}|^{(1/2)}$ and $b=a_{22}/|a_{12}a_{21}|^{(1/2)}$. Note that in general a and b are not simply parameters, but functions of the variables of the system. As for the product of the normalized nondiagonal terms, it is 1 or -1 depending on whether the 2-circuit is positive or negative. Although there is a unified expression (in which one uses the *sign* of the 2-circuit) it is convenient to treat these two cases separately.

If the 2-circuit is positive, the argument of the square root in the analytic expression of the eigenvalues is positive. The eigenvalues are thus necessarily real [in agreement with the necessity of negative 2- (or more) circuits for temporal periodicity]. The eigenvalues will be of the same or of opposite signs depending simply on whether $ab>1$ or $ab<1$. The partition is thus ensured by the pair of branches of the hyperbola described by $ab=1$. Accordingly, any steady state will be of the nature indicated by its location in space ab, the space of the normalized circuits. The situation is described in Fig. 5(a).

If the 2-circuit is negative, the argument of the square root is positive or negative depending on whether $|a-b|>2$ or $|a-b|<2$. Thus, between the straight lines $b=a\pm 2$ the eigenvalues are complex, outside these lines they are real. Within the straight lines, the sign of the real part of the eigenvalues is the sign of $a+b$. Outside the straight lines, the domains of equal and opposite signs of the eigenvalues are delimited by the pair of branches of the hyperbola described by $ab=-1$ [Fig. 5(b)].

These general diagrams permit a direct comparison between (1) the partition according to the signs of the normalized one-element circuits, which is nothing else than the partition into four quadrants by axes *a* and *b*, and (2) the partition according to the signs of the eigenvalues or their real part. A pair of branches of a hyperbola, whose orientation depends on whether the 2-circuit is positive or negative, delimits the domains. It may be noticed that the two types of partitioning coincide except for the crescents between the branches of the hyperbola and the axes.

Three- (or more) variable systems can also be normalized by an appropriate circuit or a union of disjoint circuits. This permits one to grasp in quite general terms in which way the dynamics of a system changes when a full-circuit is complemented by additional terms of increasing size. This analysis will be described in a subsequent paper.

V. FUNCTIONS OF CIRCUITS IN GENERATING PERIODICITY

In order to have stable temporal periodicity, one needs a negative circuit of at least two elements (at least in differential systems without delays). In linear systems, this only leads to spiraling inwards or outwards without stabilization on a closed trajectory. To stabilize periodicity, one needs a nonlinearity such that the real part of the eigenvalues is positive in the vicinity of a steady state but negative at some distance. Since in two dimensions the real part of the eigenvalues is simply equal to half the trace of the Jacobian matrix, this can be achieved even by a single diagonal term of appropriate structure like in the system

$$\dot{x}=x-x^3+y, \quad \dot{y}=-x,$$

whose Jacobian matrix is

$$\begin{pmatrix} 1-3x^2 & 1 \\ -1 & 0 \end{pmatrix}.$$

This generates a limit cycle, as shown by Fig. 6. The example also shows that even though stable periodicity requires both a negative two- (or more) element circuit and a proper nonlinearity, the nonlinearity does not have to be located on the circuit that generates the periodicity (here, the negative 2-circuit). As far as we can tell, the nonlinearity has to be located on a circuit; which, however, definitely does not have to be full-circuit, in contrast with the requirements for multistationarity. The two statements are demonstrated by the example above.

Periodicity and multistationarity. Consider the system

$$\dot{x}=-cx+y, \quad \dot{y}=-x+d\tanh(y),$$

whose Jacobian matrix is

$$\begin{pmatrix} -c & +1 \\ -1 & d\ \mathrm{sech}^2(y) \end{pmatrix}.$$

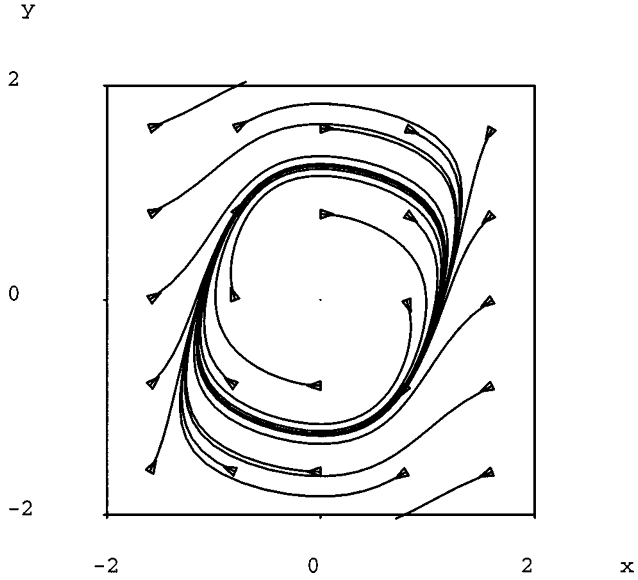


FIG. 6. $\dot{x}=x-x^3+y, \dot{y}=-x$, whose Jacobian matrix is $\left(\begin{smallmatrix} 1-3x^2 & +1 \\ -1 & 0 \end{smallmatrix}\right)$. There is a single steady state, in agreement with the fact that there is a single, nonambiguous full-circuit. The negative 2-circuit generates periodicity. Confinement is ensured by the fact that the trace is positive for small values of *x* but negative outside.

This system is illustrated by Fig. 7 for $c=0.73$ and $d=2$. In agreement with the presence of two full-circuits of opposite signs and of a negative 2-circuit, there is multistationarity and stable periodicity for proper values of the parameters. There is no ambiguous circuit since tanh is a monotonous function and its derivative $\mathrm{sech}^2(y)$ is positive everywhere. Thus, phase space is not partitioned according to the sign of any circuit. The plain horizontal lines in Fig. 7 show the partition of phase space according to the signs of the eigenvalues or their real part. In the region outside the dashed horizontal lines, the eigenvalues are complex. Accordingly, the steady state located at (0,0) is a saddle point and the outer steady states are stable foci. In addition, there is a stable limit cycle and, as kindly remarked by Holmes,[30] two unstable limit cycles (see Fig. 7).

VI. FUNCTIONS OF CIRCUITS IN DETERMINISTIC CHAOS

The role played by circuits in deterministic chaos has been analyzed in Thomas.[31–33] Suffice to mention here our

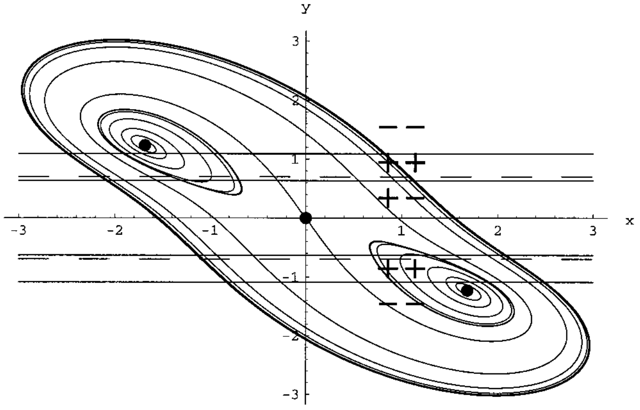


FIG. 7. $\dot{x}=-cx+y, \dot{y}=-x+d\tanh(y)$, whose Jacobian matrix is $\left(\begin{smallmatrix} -c & +1 \\ -1 & d\ \mathrm{sech}^2(y) \end{smallmatrix}\right)$, with $c=0.73$ and $d=2$. The unbroken horizontal lines correspond to the values of *y* for which the eigenvalues (or their real part) switch from + to −. Outside the dashed lines, the eigenvalues are complex.

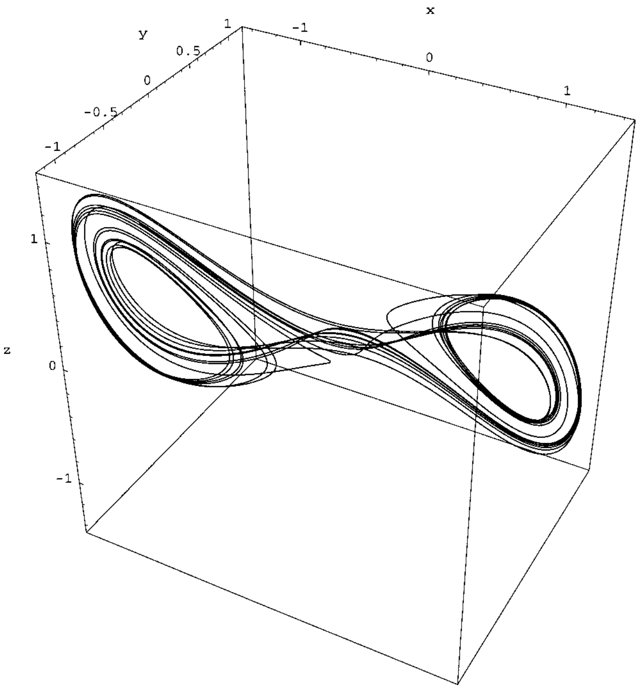


FIG. 8. $\dot{x}=cx+z, \dot{y}=x-y, \dot{z}=-x^3+y$, whose Jacobian matrix is $\begin{pmatrix} c & 0 & +1 \\ +1 & -1 & 0 \\ -3x^2 & +1 & 0 \end{pmatrix}$. There is multistationarity (three steady states, a saddle point of type $+/--$, and two saddle points of type $-/++$), in agreement with the presence of two full-circuits of opposite signs. There is a positive circuit responsible for multistationarity (from that viewpoint, the positive 1-circuit is dispensable) and a negative 2-circuit responsible for periodicity around the two external foci. For c near 0.49, the dynamics is chaotic.

provisional conclusions and show an example. In order to generate the types of deterministic chaos we have analyzed, one needs two or more coupled, yet distinct periodicities. ''Distinct'' means that they are organized around distinct (full or partial) steady states. Nevertheless, the same negative circuit can generate them. Thus, one would need at least a negative circuit with two or more elements to generate periodicity and a positive circuit to generate full or partial multistationarity. All the many (pre-existing or synthesized here) chaotic systems we checked indeed fulfill these criteria. Figure 8 shows the trajectory of the system

$$\dot{x}=0.49x+z, \quad \dot{y}=x-y, \quad \dot{z}=-x^3=y,$$

whose Jacobian matrix is

$$\begin{pmatrix} +0.49 & 0 & +1 \\ +1 & -1 & 0 \\ -3x^2 & +1 & 0 \end{pmatrix}.$$

This system, which comprises a positive 3-circuit responsible for multistationarity, a negative 2-circuit responsible for periodicities and two linear diagonal terms, displays a nice chaotic dynamics.

It is worthwhile to recall that the two types of circuit, one negative, one positive, can be provided by a single ambiguous circuit with three or more elements, so that deterministic chaos can be generated by a single ambiguous circuit with three or more elements.[33]

VII. CONCLUSIONS

Nonlinear dynamics has already been considered in terms of circuits by May,[25] by Tyson[26] and by Eisenfeld and de Lisi,[23] with a special emphasis on stability.

Probably in view of our biological background, we are less interested in the criteria for stability (or for instability) *per se* as in the conditions which lead to the two deeply divergent modalities of instability. One modality leads to multistationarity (and, in biology, to differentiation) *via* positive real roots of the characteristic equation, generating basins delimited by separatrices. The other modality leads to stable periodicity or its punctual variant (and, in biology, to homeostasis), *via* pairs of complex roots of the characteristic equation. We provide statements concerning the role of circuits in these two types of instability and their involvement in various nontrivial processes.

On the other hand, as linear stability analysis is so efficient, one might question the utility of developing another—not alternative, but rather complementary—method. One justification is that in many cases a simple look at the Jacobian matrix of a system in terms of circuits can give precious information about its dynamical possibilities. Consider, for example, the matrix

$$\begin{pmatrix} - & - & 0 \\ + & - & - \\ - & 0 & - \end{pmatrix}.$$

This matrix comprises three full-circuits, a 3-circuit, the union of a 2-circuit in *xy* and of a 1-circuit in *z*, and the union of three 1-circuits. This indicates the possibility of three types of steady states, a saddle-focus of type $-/++$, a stable focus of type $-/--$ and a stable node. However, the absence of any positive circuit precludes any multistationarity. Consequently, in this system, one can have either of these three types of steady states depending on the values of the parameters, but they cannot coexist.

We have seen that when a system is restricted to a pure full-circuit the nature of the steady state(s) can be directly derived from the signs of the elements of the Jacobian matrix. The situation is of course more complex in the presence of additional terms. However, the way in which the additional terms modify the eigenvalues can be, and has already been, partly analyzed. For this work, the normalization described in this paper is of a great help.

ACKNOWLEDGMENTS

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[1] R. Briggs and T. J. King, ''Transplantation of living nuclei from bastula cells into enucleated frog's eggs,'' Proc. Natl. Acad. Sci. U.S.A. **38**, 455–463 (1952).

[2] J. B. Gurdon, ''Adult frogs derived from nuclei of single somatic cells,'' Dev. Biol. **4**, 256–273 (1962).

[3] B. Mintz, ''Somatic cell origin of teratocarcinoma,'' Proc. Natl. Acad. Sci. U.S.A. **75**, 2834–2838 (1978).

[4] C. H. Waddington, ''The genetic control of development,'' Symp. Soc. Exp. Biol. **2**, 145–154 (1949).

[5] M. Delbrück, ''Unités biologiques douées de continuité génétique,'' Colloq. Int. C. N. R. S. **8**, 33–35 (1949).

[6] R. Thomas and R. D'Ari, *Biological Feedback* (CRC Press, Boca Raton, FL, 1990).

[7] A. Novick and M. Weiner, ''Enzyme induction as an all-or-none phenomenon,'' Proc. Natl. Acad. Sci. U.S.A. **43**, 553–566 (1957).

[8] M. Cohn and K. Horibata, ''Inhibition by glucose of the induced synthesis of the beta-galactoside-enzyme system of Eschericia coli, Analysis of maintenance, '' J. Bacteriol. **78**, 601–612 (1959).

[9] M. Cohn and K. Horibata, ''Analysis of the differentiation and of the heterogeneity within a population of Escherichia coli undergoing induced beta-galactosidase synthesis,'' J. Bacteriol. **78**, 613–623 (1959).

[10] M. Cohn and K. Horibata, ''Physiology of the inhibition by glucose of the induced synthesis of the beta-galactoside-enzyme system of Escherichia coli,'' J. Bacteriol. **78**, 624–635 (1959).

[11] H. Eisen, P. Brachet, L. Pereira da Silva, and F. Jacob, ''Regulation of repressor inhibition in lambda,'' Proc. Natl. Acad. Sci. U.S.A. **66**, 855–862 (1967).

[12] M. Ptashne, *A Genetic Switch. Gene Control and Phage Lambda* (Cell Press and Blackwell Scientific Publications, City, 1986).

[13] J. Monod and F. Jacob, ''General conclusions: teleonomic mechanisms in cellular metabolism, growth and differentation,'' Cold Spring Harbor Symp. Quant. Biol. **26**, 389–401 (1961).

[14] S. A. Kauffman, ''Control circuits for determination and transdetermination,'' Science **181**, 310–318 (1973).

[15] L. Wolpert and J. H. Lewis, ''Towards a theory of development,'' Fed. Proc. **34**, 14–20 (1975).

[16] R. Thomas, A. M. Gathoye, and L. Lambert, ''A complex control circuit. Regulation of immunity in temperate bacteriophages,'' Eur. J. Biochem. **71**, 211–227 (1976).

[17] E. Plahte, T. Mestl, and S. W. Omholt, ''Feedback loops, stability and multistationarity in dynamical systems,'' J. Biol. Syst. **3**, 409–413 (1995).

[18] E. H. Snoussi, ''Necessary conditions for multistationarity and stable periodicity,'' J. Biol. Syst. **6**, 3–9 (1998).

[19] J.-L. Gouzé, ''Positive and negative circuits in dynamical systems,'' J. Biol. Syst. **6**, 11–15 (1998).

[20] J. Demongeot, ''Multistationarity and cell differentation,'' J. Biol. Syst. **6**, 1–2 (1998).

[21] R. Thomas, ''The role of feedback circuits: positive feedback circuits are a necessary condition for positive real eigenvalues of the Jacobian matrix,'' Ber. Bunsenges. Phys. Chem. **98**, 1148–1151 (1994).

[22] J. Reignier *et al.*, in preparation.

[23] J. Eisenfeld and C. de Lisi, ''On conditions for qualitative instability of regulatory circuits with application to immunological control loops, in *Mathematics and Computers in Biomedical Applications*, edited by J. Eisenfeld and C. de Lisi (Elsevier, Amsterdam, 1985), pp. 39–53.

[24] J. Quirk and R. Ruppert, ''Qualitative economics and the stability of equilibrium,'' Review of Economic Studies **32**, 311–326 (1965).

[25] R. M. May, *Stability and Complexity in Model Ecosystems* (Princeton University Press, Princeton, 1974).

[26] J. Tyson, ''Classification of instabilities in chemical reaction systems,'' Chem. Phys. **62**, 1010–1015 (1975).

[27] M. Cahen (private communication).

[28] D. Carlson, ''Nonsingularity criteria for matrices involving combinatorial considerations,'' Linear Algebr. Appl. **107**, 41–56 (1988).

[29] J. J. McDonald, D. D. Olesky, M. J. Tsatsomeros, and P. van den Driessche, ''Ray patterns of matrices and nonsingularity,'' Linear Algebr. Appl. **267**, 359–373 (1997).

[30] P. Holmes (private communication).

[31] R. Thomas, ''Analyse et synthèse de systèmes à dynamique chaotique en termes de circuits de rétroaction (feedback circuits),'' Bull. Cl. Sci., Acad. R. Belg. **7**, 101–124 (1996).

[32] R. Thomas, ''The Rössler equations revisited in terms of feedback circuits,'' J. Biol. Syst. **7**, 225–237 (1999).

[33] R. Thomas, ''Deterministic chaos seen in terms of feedback circuits: analysis, synthesis, 'labyrinth chaos,' '' Int. J. Bifurcation Chaos Appl. Sci. Eng. **9**, 1889–1905 (1999).