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Laws for the dynamics of regulatory networks

RENÉ THOMAS*

Laboratory of Genetics, Université Libre de Bruxelles, Rhode St Genèse, Belgium

ABSTRACT We start our analysis from historical but too seldom quoted papers by Delbrück, Novick & Weiner, Cohn & Horibata and Monod & Jacob. We try to show how it became possible to draw a line coupling cell differentiation to the physical concept of multistationarity, and the latter to the concept of positive feedback circuits. Two laws give the minimal logical ingredients required for differentiative and homeostatic regulations. It is briefly shown how they can be used to treat such complex dynamics as deterministic chaos, which, admittedly, does not yet belong to the corpus of developmental biology. It was taken as a challenge to express our ideas here in purely verbal terms, avoiding any formal treatment.

KEY WORDS: *feedback, logical structure, homeostasis, determination*

Epigenetic differences

Epigenetic differences are those which can be transmitted from a cell to its progeny in the absence of any genetic difference. They are often ill-perceived. In particular, it is sometimes surmised that "epigenetic" must be opposed to "genetic"; in fact, as we will see, epigenetic differences can be found only in the presence of an appropriate genetic background.

A good way to introduce epigenetic differences is to describe the admirable experiences of Novick and Weiner (1957) and of Cohn and Horibata (1959a,b,c). It was known from the work of Monod and his team that the genes comprised in what would be later called "the lactose operon" are expressed only in the presence of a small molecule, related to lactose itself and denoted "inducer". One of these genes codes for a specific "permease" which actively pumps the inducer (as well as lactose itself) from outside to inside the bacterial cell. Cells in which the lactose genes are expressed are called "induced".

An essential point is that there is a wide range of extracellular concentrations of inducer which are not sufficient to establish the induced state but are sufficient to maintain it if it is already present. Now, the experiments can be described in a simplified way as follows. Take an uninduced culture of *E. coli*. Add a high amount of inducer, immediately split the culture into two parts (say A and B) and dilute them so that the extracellular concentration of inducer drops to reach the "maintenance" range. The only difference between subcultures A and B is that in A dilution took place immediately whereas in B the experimenter waited for ten minutes or so. Yet, subculture A, which was not in contact with a high concentration of inducer for a significant time, is and remains uninduced. In contrast, subculture B is and remains induced, because it was in contact with a high concentration of inducer for enough time to be fully induced, and dilution to the maintenance concentration does not change the situation.

To keep them growing, subcultures A and B can be serially diluted, always in the same medium (with the maintenance concentration of inducer). This was done for 150 generations, after which A remained uninduced, B fully induced. The results were made even more striking by the demonstration that when a mixture of uninduced and induced cells is present in «maintenance» medium, the progeny of the induced (vs. uninduced) cells is induced (vs. uninduced).

This is, no doubt, one of the most beautiful examples of epigenetic differences: there are two cultures which are obviously genetically identical (and can easily be shown to have remained so) and grow in identical external conditions, yet display different phenotypes for many cell generations. The only difference is a "detail" of their previous history: a transient signal (a brief exposure to high concentrations of inducer) resulted in a lasting and transmissible phenotypic change.

The mechanism of the process was perfectly understood by the authors. It can be schematically described as follows (Fig. 1). The inducer is required intracellularly for the synthesis of permease, and at the concentration of extracellular inducer used in the experiment the penetration of inducer requires permease. Thus, a cell which does not contain any permease molecule will be unable to pump inducer in, and consequently to produce any permease molecule: a vicious circle. In contrast, a cell already containing even a single molecule of permease will concentrate inducer and produce more permease, thus rendering the synthesis permanent.

***Address for reprints:** Laboratory of Genetics, Université Libre de Bruxelles, 67, rue des Chevaux, 1640 Rhode St Genèse, Belgium. FAX: 32-2 650 97 44. e-mail: rthomas@dbm.ulb.ac.be

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Epigenetic differences are the biological facet of a more general process, called multistationarity by physicists

Some years before the Novick-Weiner and Cohn-Horibata experiments, most biologists where convinced that the differences between cell lines within an organism were due to the presence of different populations of "plasmagenes". Plasmagenes were supposed to be particles produced by the genes and carrying their genetic information away (like the future messenger RNA). These particles were thought to be released into the cytoplasm where they not only expressed themselves, but also replicated (unlike the future messenger RNA). In a brief comment to a paper by Sonneborn and Beale (1949), based on the concept of plasmagenes, Delbrück (1949) suggested an alternative view: epigenetic differences, including those involved in differentiation, might be the biological aspect of a more general process, multistationarity. In his paper, published in French translation, the process is called "équilibres de flux". We gave elsewhere a re-translation of Delbrück's paper, in which we took the liberty of translating "équilibres de flux" as "multiple steady states" (Thomas and D'Ari, 1990). The appropriateness of this choice was confirmed when we obtained the original manuscript of Delbrück (written in English), thanks to the courtesy of Maurice Fox.

But what exactly is meant by "multistationarity" and "multiple steady states?" There exist systems whose structure is such that in exactly identical external situations they can have two or more distinct steady states (defined as states in which the time derivative of each variable is nil). This forces many of these systems to a choice between two or more lasting regimes, each of which may be static, periodic or more complex according to the stability properties of the steady states. The example given by Delbrück was purely theoretical, but the above-mentioned experiments and many others have since given his idea a firm foundation. It has since become clear that epigenetic differences, including those involved in differentiation, are typical cases of multistationarity.

Just afterwards, in a prophetic but unfortunately seldom quoted paper, Monod and Jacob (1961) proposed several theoretical models to account for epigenetic differences and differentiation in this type of context (this paper is not to be confused with the well-known founder paper of biological regulation, Jacob and Monod, 1961). They mention the connection with feedback, use the term autocatalysis for one of their models and, unlike most more recent papers, quote Delbrück as well as Novick and Cohn. The role of feedback in differentiation is also explicitly mentioned in an important paper by Wolpert and Lewis (1975).

One crucial element is common to the various mechanisms (theoretical or experimental) mentioned above: the presence of a *positive* feedback circuit [see for example Thomas *et al.* (1976)]. But in order to justify this statement, it is first necessary to comment briefly on biological regulatory networks and introduce feedback circuits.

Biological regulation and the shape of regulatory interactions

When one thinks of biological regulation, one almost always refers to the mechanisms involved in homeostasis, which maintain our body temperature, our blood pressure or the concentration of a hormone near a supposedly optimal level, and far from the extreme values which would prevail if the system were functioning either fully or not at all. These mechanisms operate like a thermostat, with or without oscillations according to the case.

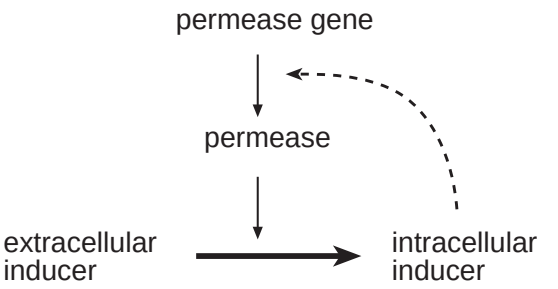

Fig. 1. The lactose "differentiative" system.

In contrast, there is another type of regulation which obliges the system to choose one of these extreme values. At first view, such a mechanism might seem absurd because it resembles a modified thermostat which would light the heater when the temperature is already high and switch it off when the temperature is low! However, such a device has the merit that it permits a system to switch a process on or off as the result of a signal. This is precisely the type of regulation which is required to account for epigenetic differences, including cell differentiation, and accordingly we call it "differentiative" regulation.

Biologists are very familiar with the concept of homeostasis. In contrast, the alternative type of regulation is seldom correctly perceived, partly because it is commonly felt that it only gives a choice between "nothing" and "explosion". However, almost all biological processes saturate, and instead of "nothing or explosion", the differentiative type of regulation offers a choice between two extreme levels (such as "gene on" vs. "gene off").

Biological regulations are ensured by networks whose basic elements will be analyzed below. It is, however, a prerequisite for a reasonably coherent description of these processes first to say something about the interactions involved.

It is crucial to realize that regulatory interactions are almost always nonlinear. This simply means that the rate of the controlled process is not a linear function of the concentration of the regulator. Most biological regulatory interactions are sigmoid in shape (Fig. 2B). Consider, for example, a gene whose expression depends on the presence of a positive regulator. Typically, for increasing concentrations of the regulator, the rate of expression of the gene is first insignificant, then sharply raises within a rather narrow range, and finally levels off.

As regulatory interactions are nonlinear, the differential equations used for their description are themselves nonlinear and can in general only be integrated numerically. For this reason, it is tempting to use idealizations. The most obvious one consists of a linear approximation (Fig. 2A). This simplification is valid in the close vicinity of steady states, but disastrous elsewhere. As the nonlinearities commonly found in biological regulatory processes are sharply sigmoid in shape, another idealization is to simplify them as step functions (Fig. 2C). The so-called "logical" descriptions, in their elementary versions, reason as if a gene product were either "absent" (= below threshold), or "present" (= above threshold), and the gene, "off" or "on".

It turns out that this type of caricature results in a representation that retains, at least qualitatively, the essential features of the

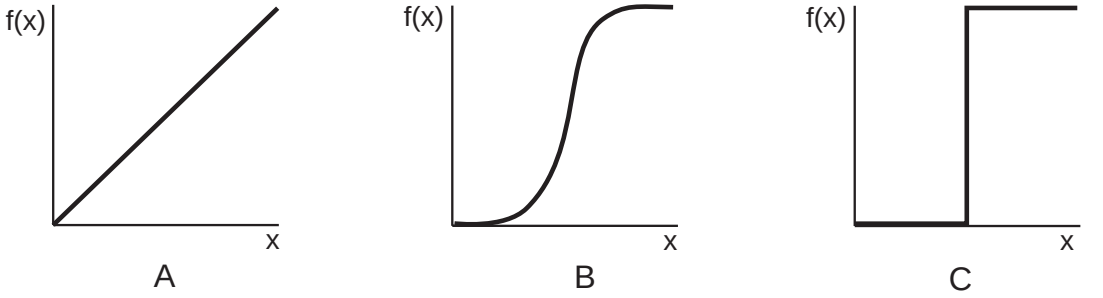

Fig. 2. A non-linear (sigmoid in this case) interaction, B, simplified as linear (A) and step (C) functions. *Concretely, f(x) is the rate of expression of a regulated gene, plotted as a function of x, the concentration of a positive regulator.*

dynamics of the original system, hence the recent success of these "logical" descriptions (Kauffman, 1969, Thomas, 1973).

Positive and negative feedback circuits

Although biologists usually use the term "feedback loop", we prefer "feedback circuit" because graph theoreticians reserve the word "loop" for circuits comprising a single element. The concept of oriented feedback circuit (here simply called "circuit") has been used for many years by biologists, electromechanics and ecologists. One says that elements A, B and C form a circuit if the level of A exerts an influence on the rate of production of B, whose level influences the rate of production of C, whose level in turn influences the rate of production of A (note that while some interactions are explicitly regulatory, such as the inhibition of an early step by the end product of a metabolic chain, or the repression or activation of gene expression by a regulator, other interactions may be less obviously regulatory, for example those which consist of the conversion of a metabolic intermediate into the next one). In a circuit, each element exerts a direct action on the next element in the circuit, and also (except, of course, for one-element circuits) an indirect effect on all other elements, including itself.

There are two classes of feedback circuits. Either each element in the circuit exerts a positive action (activation) *on its own* future evolution, or each element in the circuit exerts a negative action (repression) on this evolution. It is thus natural to call these circuits positive and negative, respectively. Whether a circuit is positive or negative simply depends on the parity of the number of negative elements in the circuit: a circuit with an even number of negative elements is positive, while if this number is odd the circuit is negative.

The properties of the two types of circuits are strikingly different; in fact, they are the logical bases underlying the two types of biological regulation described above. A negative circuit functions like a thermostat and generates homeostasis (with or without oscillations). In contrast, a positive circuit can force a system to choose lastingly between two extreme situations, as is the case in differentiation and, more generally, in systems with multiple steady states. Thus, the essential properties of negative and positive feedback circuits are precisely those expected for the mechanisms which ensure the two types of biological regulations, homeostatic and differentiative, respectively.

The contrasted behavior of the two types of circuits can be justified without difficulty if one formalizes the circuits in terms of systems of ordinary differential equations or by "logical" methods (Thomas and D'Ari, 1990). In this paper, however, I will try to describe the behavior of circuits in purely verbal terms, taking first the concrete situation of gene control.

Behavior of negative circuits

Consider a gene whose product directly or indirectly represses its own synthesis (a typical negative circuit). Intuitively, one expects that as the concentration of the product increases, its rate of synthesis will decrease; and since a gene product is subject to decay and/or dilution, its concentration will decrease, resulting in release of repression, and so on. Depending on the parameters of the system, such a situation may result in sustained oscillations around a mean value, or lead to a stable mean value with the gene "half-on, half-off", with or without transient oscillations. The same type of reasoning can be made for any negative circuit, whatever its number of elements. Thus, a gene subject to direct (one-element circuit) or indirect negative control can be "half-on, half-off", or it can oscillate, depending on parameter values. Note that if one represents circuits by the signs of their constituent interactions, a one element negative circuit is (-), a two-element circuit is (+ -) or (- +), a three-element circuit, (- - -), (- + +), (+ - +) or (+ + -), etc.

In the differential description, only negative circuits with two or more elements (and proper parameter values) can give rise to oscillations. In practice, even when we reason in terms of a gene regulated by its own product, and formalize it as if it were a one-element circuit, we are actually dealing with at least a two-element circuit, if only because the gene product, usually a protein, is synthesized *via* a m-RNA. In addition, when a gene is switched on, it may take one minute or so before the very first molecule of m-RNA is completed, and thereafter up to several minutes before the very first molecule of protein is synthesized. If one takes the occurrence of such absolute time delays into account, and introduces them into the differential equations, even one-element negative circuits can oscillate.

Negative autoregulation is extremely frequent in biology. It is not surprising that it has often been selected for, since it is the simplest way to maintain the level of a gene product near its threshold value at the lowest possible cost. In addition, negative autoregulation is an efficient way of buffering the effects of gene dosage (see Thomas and D'Ari, 1990).

Behavior of positive circuits

Consider now a gene whose product exerts a positive control on its own synthesis. To facilitate a verbal analysis, we will first focus on the ideal situation of a gene whose product would be both necessary and, in normal conditions, also sufficient for its own synthesis. In the absence of the product, the gene will be and remain off. In the presence of the product, the gene will be on and, since it is on, more product will be synthesized and the gene will

remain on. Thus, the occurrence of this one-element positive circuit (sometimes also called "direct autocatalysis") suffices to account for the fact that a gene can persist in either of two stable states, "on" and "off", in the same environment. Strictly speaking, the formal analysis shows that (at least for sigmoid interactions, which are by far the most frequent in biological systems) a positive circuit usually generates three steady states, only two of which are stable.

A gene subject to direct positive autoregulation (+) is either on or off**.** In a positive circuit comprising two elements, the expression of the two genes involved may be interdependent or, on the contrary, exclusive. For a circuit comprising two interdependent genes (+ +), the two possible steady situations are both genes on, and both genes off. For a circuit comprising two mutually exclusive genes (- -), either gene *X* is on and gene *Y* is off, or else gene *X* is off and gene *Y* is on. The intuitive feeling that these situations might generate an oscillatory expression of the genes is widespread but perfectly erroneous, as shown both by differential and asynchronous logical analyses. For positive circuits with more than two elements, there are also two alternative states of regime, such as 0110 vs. 1001 for a (- + - +) circuit.

In one-element positive circuits, however, we have no idea of why the gene is on or why it is off, nor of how to switch it from "on" to "off" or vice versa. The origin of the decision lies outside of the circuit: whenever a gene exerts a direct positive action on its own expression, it is usually switched on by another product and the autoregulation serves to maintain it on. The well-documented case of the lambda gene *cI* is described in the next section.

We mentioned above that the concept of homeostasis is much more widely perceived than that of epigenetic difference. No surprise then that negative circuits are usually better understood and much more often alluded to than positive circuits. We have therefore two reasons for treating positive circuits more extensively here: because they are less widely understood, and because they are crucial for differentiation.

Positive feedback and differentiation

When asked why the gene coding for serum albumin is expressed in liver cells but not in, say, intestine cells, it is tempting to answer that it is because liver cells contain a transcription factor that is absent in intestine cells, or because intestine cells contain a repressor absent in liver cells. These assumptions are certainly reasonable. However, if one now asks why liver cells would contain a transcription factor absent from intestine cells, it is realized that even though the answer to the first question may be correct, it only displaces the problem. This is reminiscent of the explanation of the structure of the universe in which the earth is supported by an elephant, whose members are each supported by a tortoise, which in turn etc. In addition, why is it that this situation is *stably* maintained from generation to generation?

In view of the preceding paragraphs, the reader will not be surprised by our suggestion to close the causality on itself, by introducing a positive feedback circuit. Clearly, if the gene coding for albumin were controlled, positively or negatively, by a gene which exerts a direct or indirect positive control on its own expression, the albumin gene would be locked either in "on" or in "off" position depending on the previous history of the cell, and this situation would be transmissible through cell division.

One of the very first documented examples of this type of positive circuit is that of immunity in temperate bacteriophages. These viruses can persist in two radically different ways. Either they multiply as classical viruses and kill their host cell, or they establish a close symbiotic association, called lysogeny**,** with their host**.** In the classical case (lambda and "lambdoid" phages), the viral chromosome is integrated into the host chromosome (under the name "prophage") and transmitted as such to the bacterial progeny. However, in this condition the viral genes are harmless because one of them (gene *cI)* produces a repressor which prevents all the lethal viral genes from being transcribed (Jacob and Monod, 1961). This situation is called immunity because it protects a lysogen not only against the prophage it carries, but also against infection with an extrinsic phage of the same specificity.

When a bacterial population is infected with a temperate bacteriophage, part of the cells lyse and produce more virus, part survive and generate a lysogenic progeny. There are even strains, carrying an impaired prophage, which can indefinitely persist in either of two states, with or without immunity. But how is it that the bacterial population can behave in two such different ways, in other words, differentiate? This in fact amounts to asking how gene *cI* is regulated.

It turns out that gene *cI* is positively regulated by its own product (Eisen *et al.,* 1967) -a positive circuit. If it were dependent on its own product only, the situation would be a vicious circle in the sense that it would be stably on or stably off depending on whether or not some cI product was already present; thus, one would understand that it can be stably on or off, but not how one of these possibilities has been selected. In fact, gene *cI* is also under positive control of gene *cII* (Kaiser and Jacob, 1957). In the absence of both products, gene *cI* is and remains off. As soon as the *cII* product is present, gene *cI* is switched on, it synthesizes its product which activates is own synthesis, and from now on gene *cI* remains on, whether the *cII* product is present or not. As a matter of fact, the *cI* product represses all the other genes, including its own "lighter", *cII*, so that very soon after gene *cI* has been switched on it switches off gene *cII*. The expression of *cI* remains on, however, because of its "autocatalytic" character.

The positive action of gene *cI* on itself accounts for the fact that it can persist in either of two stable states, on or off. Thus, a simple positive circuit can not only generate a choice between alternative steady states, but in addition it can transform the effect of a transient signal into a permanent change: positive circuits provide memories of signals, they convert their transient occurrence into permanent change. This is the basis of the phenomenon of perdurance (García-Bellido and Merriam, 1971): once a signal has produced its effect, the corresponding gene can be removed from the cell without any harmful effect on further development, because the effect of the signal can be maintained indefinitely through many cell divisions. One of the earliest examples of a developmental memory effect due to a positive circuit was demonstrated in the case of the *Sxl* gene, which controls sex determination in *Drosophila* (Cline, 1984).

Many steady states can be generated by several positive circuits

In order to account for many cell types in terms of multiple steady states, many steady states are needed (Monsieur de la Palice, personal communication). But how can one obtain many steady states? At first view, one might have thought that the

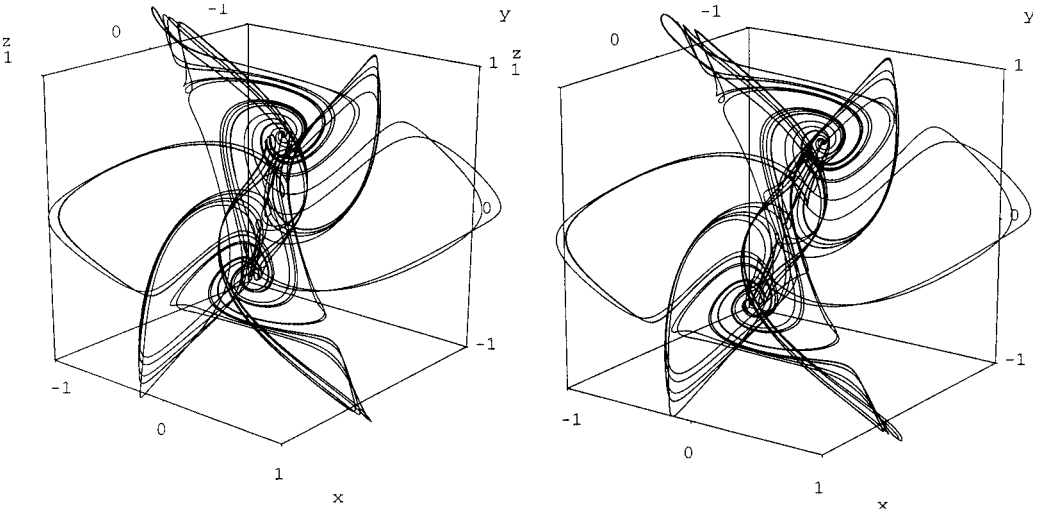

Fig. 3. A "strange attractor" (in stereoscopic view) developed as an answer to the question: "Can one build a system that comprises a single, three-element feedback circuit (with appropriate diagonal terms) and yet can display chaotic dynamics?" *The answer is "yes", provided the circuit can be positive or negative depending on the location in phase space.*

number of steady states generated by a positive circuit would depend on the number of its elements. This is not the case; provided the interactions are sigmoid in shape, as is usually the case in biological systems, a positive circuit typically creates a choice between two permanent regimes, whatever its number of elements.

The simplest way to have many steady states is therefore to use several positive circuits. Consider a biological system of, say, 8 regulatory genes, each of which exerts a (direct or indirect) positive control on its own expression. For proper parameter values, each of these genes can be stably on or off independently of the others, so that such a system can define up to 2^8= 256 possible cell types, each characterized by the lasting presence or absence of the proteins whose synthesis is regulated by these eight genes. More generally, at least in the case of sigmoid or step interactions, 'n' isolated positive circuits typically permit a choice between 2^n stable situations.

There may of course be interactions between the circuits considered. This usually tends to lower the number of steady states, unless the additional interactions create additional positive circuits. Furthermore, some of the genes considered will also presumably be involved in negative circuits. For example, if gene *X* activates the first member of a (+ -) loop, then when gene *X* has been off for some time, the other two genes will be off as well, whereas if gene *X* is on, the other two genes can be half-on and half-off, or oscillate. Finally, each regulatory gene will usually regulate several other genes, thus being pleiotropic.

The idea that the decision made at the level of a positive circuit is permanent might be taken to mean that it is irreversible. In fact, whenever the expression of a gene depends on more than one factor, the situation can be lastingly modified as a result of a new signal, transient or permanent. Furthermore, we would like to stress that the decision made at the level of a positive circuit usually does not lead directly to a stable regime, but rather does it via a sequence of intermediate states. It would thus be more general, and perhaps less naive, to say that the decision has resulted in a choice between two or more distinct sequences of states rather than to focus on the final stable states only. Presumably, many cell types observed during development correspond to these situations, called "transitories" by physicists.

More complex networks

In the hope of being clear about the fundamental principles, we have so far alluded to very simple systems only, comprising one or few circuits and displaying elementary behavior. At this level of low structural and dynamical complexity, it would be perfectly justified to question the interest of a formal description, and to ask whether it is actually required to handle such simple systems. But of course, the biological reality involves complex networks comprising intertwined circuits and displaying extremely complex behavior. It is precisely at this level that the approach based on feedback circuits finds its full justification.

One of the conclusions that we have reached from this approach is that, however complex a network may be, it can always be decomposed into individual circuits. An essential point is that each of these individual circuits, no matter how much it may be connected to other individual circuits within the network, keeps its individuality and can be identified and characterized as such (see for example Thomas and D'Ari, 1990; Thomas, 1991; Thomas *et al.,* 1995). Of course, whether and how they operate depends on their interactions with other elements of the network. As a result, it has become possible to proceed as follows: first identify and characterize the individual circuits, then compute the range of logical parameters within which each circuit or set of circuits is functional, taking into account the interference from other circuits, with the aim of deriving a global view of the possible dynamics of the network. This amounts to treating a network as the set of its interacting circuits rather than as the set of all of its individual elements. If we may use a metaphor, it is somewhat like first taking into account the cogwheels of a clock rather than the individual cogs which constitute these wheels.

To be honest, even though the use of such methods permits one to treat systems very significantly more complex than those accessible by more classical methods, it remains true that the complexity of the analysis increases explosively with the number of variables involved. However, as discussed in the next paragraph, systems of moderate logical complexity can display exceedingly complex behavior and are therefore full of interest.

Although biological processes usually depend on a great number of variables, we are convinced that in most cases it will sooner or

later be possible to understand their essential qualitative features in terms of a small number of crucial variables. This view stems from the observation that simple logical structures can generate complex behavior. In this context, I would like to allude briefly to our recent work, even though its relation to biology may seem futile. Deterministic chaos is a highly complex dynamics which can be generated by sets of nonlinear differential equations. These equations are sometimes surprisingly simple, but nevertheless cannot be solved analytically. Numerical integration results in often esthaetically admirable trajectories called "strange attractors", whose exact profile, though not their general shape, is extremely sensitive to initial conditions. A major interest of these trajectories (which in spite of their name have nothing in common with disorder), is that they are at the same time perfectly determined by the set of differential equations and yet perfectly unpredictable at long term. Figure 3 gives an example of a chaotic attractor whose conception was entirely based on the properties of positive and negative feedback circuits. Whether deterministic chaos will ever have a biological counterpart is an open question. Its occasional occurrence is conceivable, however, since some well-documented sets of gene interactions are more complex than the logical structures required to generate deterministic chaos. I feel that this dynamics can be viewed as a generalized type of homeostasis (with more freedom) and as such, should it occur in a biological system, it might be selected for.

Conclusions: logical structure and laws of regulatory circuits

1) Organized systems such as living organisms or cells require that the level of crucial elements somehow be evaluated and taken into account to determine their future rate of production. This is carried out by feedback circuits, which are closed, oriented chains of interactions. *Feedback circuits are either positive or negative depending on the parity of the number of negative interactions they comprise.* Feedback circuits are the wheels that control regulatory networks.

2) As suggested by Delbrück (1949) and amply substantiated since by experiment, epigenetic differences, including those involved in differentiation, are the biological facet of a more general process -multistationarity. It was conjectured long ago (Thomas, 1980) and formally proven since (Plahte *et al.,* 1995; Gouzé, 1998; Snoussi, 1998) that *(law I) a positive circuit is a necessary condition for multistationarity.* We conclude that positive circuits are a necessary condition for epigenetic differences, including those involved in differentiation.

3) It was also conjectured (Thomas, 1980) and more recently formally demonstrated (Gouzé, 1998; Snoussi, 1998) that *(law II) a negative circuit is a necessary condition for stable periodicity.* The biological corollary of this second law is that homeostasis (with or without oscillations) is based on the operation of negative circuits. Another role of negative circuits is to buffer gene dosage effects.

4) Biological regulation can rarely be described, even in a caricatural way, by a single feedback circuit. In fact, one usually faces more or less complex networks comprising intertwined circuits. In view of their crucial role, it is legitimate (and convenient) to call the ensemble of the circuits the "logical structure" of the system. *In complex networks, each individual circuit keeps its individuality and can still be identified and characterized.* However, its actual functionality depends critically on its interactions with other circuits. We now have the tools required for fully taking these interactions into account.

5) Even though biological processes usually depend on a large number of variables, we are convinced that in most cases it will sooner or later be possible to understand their essential qualitative features in terms of a small number of crucial variables. This is because *simple logical structures can generate complex behavior*. As an illustration of this point, it may be relevant to mention that the logical bases of deterministic chaos can be analyzed (and systems with chaotic dynamics, synthesized) in terms of simple networks of feedback circuits.

Epilogue

Most of the ideas developed in this paper have been published before, some of them long ago. However, as kindly reproached by my friend and former disciple Alain Ghysen, I have apparently always spoken to biologists as a physicist (which unfortunately I am not!). For the first time, I have tried to speak here as a biologist (which I have been for some decades).

The challenge of this paper was to try to express the logical rules that govern regulatory circuits verbally, without using any formalism. This is in no way a repudiation of my theoretical work: almost none of the ideas presented in this paper could have been expressed in verbal terms if it had not been previously found and developed in a rigorous way, with the help of formal methods. I started many years ago with a verbal description of biological regulation and after a long detour via formal methods, come back to a verbal description. But the present views have little in common indeed with their starting point. There is thus, as in the case of determination, a kind of hysteresis. What is, I feel, rewarding, is that it has become possible to formulate general laws concerning the relation between structure and function in regulatory networks. I have been told that these are not biological laws because their range of application is not limited to Biology. But is this really a drawback for a law to have too wide a domain of validity?

Appendix: what is and what is not feedback?

Feedback circuits are described in a loose way in the introduction. For a serious study, not only feedback circuits, but also their constituents, here called "interactions", should be defined in a more rigorous way. One reason is that not all interactions are regulatory, and there might be some ambiguity as to which of them should and which should not be taken into account.

Consider a system where substance A can be converted into substance B, and B activates this conversion. This situation is known as "product activation". The first attitude would be to focus on the only properly regulatory interaction and say that B exerts positive feedback on its own production. An alternative way consists of trying to uncover all the interactions involved, whether or not of an explicit regulatory nature. In this view, one can remark that A exerts a positive effect on B (by being converted into it) and that B exerts a negative action on A (by activating its conversion into B) and a positive action on itself (for the same reason). This "logical" analysis, by taking all interactions into account, reveals the presence of two feedback circuits: a two-element negative circuit and a one-element positive circuit.

Whenever a system can be described in terms of differential equations, one can get rid of any ambiguity in whether to call interactions positive or negative, and also in defining the identity, number and sign of the circuits. It suffices to consult the jacobian matrix of the system, i.e., the matrix of its partial derivatives. Concretely, if the term (denoted aij) located at the intersect of line 'i' and column 'j' of this matrix is non-zero, it means that element 'j' interacts with element 'i', and this interaction is positive or negative depending on whether the term itself is positive or negative. Thus, which elements of the system influence which, and in what way, can be directly read from the jacobian matrix. Circuits can also be rigorously identified as follows: a set of terms of the jacobian matrix forms a circuit (or a union of disjoint circuits) if and only if the sequence of their 'i's' and the sequence of their 'j's' are circular permutations of each other.

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