From simple to complex oscillatory behavior in metabolic and genetic control networks

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We present an overview of mechanisms responsible for simple or complex oscillatory behavior in metabolic and genetic control networks. Besides simple periodic behavior corresponding to the evolution toward a limit cycle we consider complex modes of oscillatory behavior such as complex periodic oscillations of the bursting type and chaos. Multiple attractors are also discussed, e.g., the coexistence between a stable steady state and a stable limit cycle (hard excitation), or the coexistence between two simultaneously stable limit cycles (birhythmicity). We discuss mechanisms responsible for the transition from simple to complex oscillatory behavior by means of a number of models serving as selected examples. The models were originally proposed to account for simple periodic oscillations observed experimentally at the cellular level in a variety of biological systems. In a second stage, these models were modified to allow for complex oscillatory phenomena such as bursting, birhythmicity, or chaos. We consider successively (1) models based on enzyme regulation, proposed for glycolytic oscillations and for the control of successive phases of the cell cycle, respectively; (2) a model for intracellular Ca^{2+} oscillations based on transport regulation; (3) a model for oscillations of cyclic AMP based on receptor desensitization in *Dictyostelium* cells; and (4) a model based on genetic regulation for circadian rhythms in *Drosophila*. Two main classes of mechanism leading from simple to complex oscillatory behavior are identified, namely (i) the interplay between two endogenous oscillatory mechanisms, which can take multiple forms, overt or more subtle, depending on whether the two oscillators each involve their own regulatory feedback loop or share a common feedback loop while differing by some related process, and (ii) self-modulation of the oscillator through feedback from the system's output on one of the parameters controlling oscillatory behavior. However, the latter mechanism may also be viewed as involving the interplay between two feedback processes, each of which might be capable of producing oscillations. Although our discussion primarily focuses on the case of autonomous oscillatory behavior, we also consider the case of nonautonomous complex oscillations in a model for circadian oscillations subjected to periodic forcing by a light-dark cycle and show that the occurrence of entrainment versus chaos in these conditions markedly depends on the wave form of periodic forcing. © *2001 American Institute of Physics.* [DOI: 10.1063/1.1345727]

Simple periodic behavior underlies the operation of a large number of biological rhythms. Besides neuronal and cardiac oscillations, these rhythms originate at the cellular level from regulation exerted on enzymes, receptors, transport processes, or gene expression. Complex oscillations in the form of bursting or chaos, or the coexistence between a stable steady state and stable oscillations (hard excitation), or between two stable oscillatory regimes (birhythmicity) can also occur as a result of such regulatory processes. The transition from simple to complex oscillatory behavior can be investigated in detail in models proposed for cellular rhythms. These models show that simple periodic behavior can often be associated with a particular feedback process that produces an instability beyond which the system evolves toward a limit cycle. The occurrence of complex oscillatory phenomena often involves the interplay between at least two such instability-generating mechanisms, each of which can be associated with a particular feedback process. This paper focuses on transitions from simple to complex oscillatory phenomena in metabolic and genetic control networks. The mechanisms underlying such transitions are examined in models for a variety of rhythmic processes including oscillatory enzyme reactions, cyclic AMP signaling in *Dictyostelium* cells, Ca^{2+} oscillations, the cell division cycle, and circadian rhythms.

I. INTRODUCTION

Periodic oscillations are observed at all levels of biological organization, with periods ranging from a fraction of a second to years.[1,2] A large proportion of biological rhythms are already observed at the single cell level. Thus, even if oscillations can originate from network properties in some

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neural systems, periodic trains of action potentials can be generated by a single neuron, while circadian rhythms of about 24 h period are observed in many unicellular organisms or in single cells isolated from multicellular organisms. Experimental and theoretical studies have uncovered, to a large degree, the mechanisms that underlie most of these oscillatory phenomena. In concert with nonlinearities associated with cooperative phenomena which abound in biological systems, feedback processes, of a positive and/or negative nature, appear to be involved in the origin of rhythmic behavior. Thus, cellular rhythms of various periods are associated with the regulation of enzyme activity, receptor function, transport processes, or gene expression for rhythms of a nonelectrical nature, and with the regulation of ionic conductances in electrically excitable cells.

Besides simple periodic oscillations, which represent the most prominent type of rhythm encountered in biological systems, more complex modes of oscillatory behavior can also be observed. Thus, oscillations may still be periodic but acquire a complex form, with several maxima per period: to this sort of oscillations belongs the bursting behavior characteristic of many types of nerve cells which produce several action potentials during an active phase, separated from the next bursting phase by a silent phase. Oscillations may also become irregular and aperiodic, owing to the appearance of chaos. Finally, several, simultaneously stable periodic attractors may coexist. The coexistence of two stable oscillatory regimes of different amplitude and period, known as birhythmicity,[3] is the oscillatory counterpart of the more common mode of bistability in which two stable steady states coexist in the same set of conditions.

The transition from simple to complex oscillatory behavior has quite naturally been investigated in less detail than the onset of simple periodic oscillations. Because complex oscillatory processes are also known experimentally and may play important physiological roles or disturb the function of simple periodic behavior, it is crucial to determine the mechanisms that lead from simple periodic oscillations to more complex modes of oscillatory behavior, including chaos.

The purpose of this paper is to examine a variety of mechanisms leading from simple periodic oscillations to complex oscillatory behavior and to illustrate them by examples pertaining to the dynamics of metabolic and genetic control networks. A common route to complex oscillations consists in driving periodically an oscillatory system. We shall not consider this situation (except briefly in Secs. V and VI) and will focus on autonomous systems for which simple or complex oscillations arise in the absence of any periodic clue in their environment.

We start in Sec. II by examining how the interplay between two endogenous oscillatory mechanisms can enrich the repertoire of oscillatory behavior. Two illustrative examples are considered in turn. First, the coupling in series of two oscillatory enzyme reactions of the sort responsible for glycolytic oscillations is shown to produce bursting, birhythmicity and chaos, in addition to simple periodic behavior. Second, a model of two biochemical oscillators coupled through mutual inhibition will be discussed. This model, proposed for the control of successive phases of the eukaryotic cell cycle, admits periodic, antiphase oscillations as well as chaos. Another route to complex oscillations and chaos is illustrated in Sec. III by a model for Ca^{2+} signaling in which a control parameter modulating the oscillatory dynamics is itself linked to one of the oscillatory variables of the system.

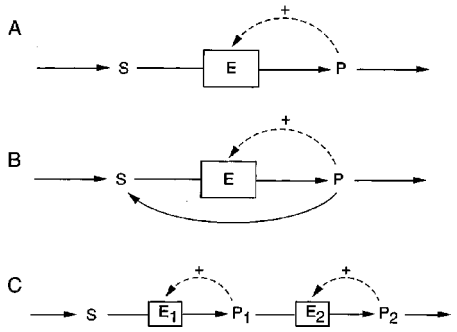

FIG. 1. Models for oscillatory enzyme reactions (see Ref. 2). (A) The product-activated enzyme reaction provides a prototype for simple periodic oscillations of the limit cycle type. (B) When product recycling into substrate is added to the previous model, the coexistence between two simultaneously stable limit cycles (birhythmicity) can be observed. (C) When two product-activated enzyme reactions are coupled in series, bursting and chaos can be observed, as well as the coexistence between a stable steady state and a stable limit cycle or between up to three simultaneously stable limit cycles.

In Sec. IV we turn to a model for cyclic AMP (cAMP) signaling in *Dictyostelium* and show that complex oscillatory phenomena arise in this system from the interplay between two oscillatory pathways sharing the same positive feedback loop in cAMP synthesis but differing by the process limiting self-amplification; this limiting process is either substrate depletion or receptor desensitization. A related mechanism considered in Sec. V underlies the occurrence of autonomous chaos and birhythmicity in a model for circadian rhythms based on the regulation of gene expression. Here, complex oscillations originate from the dynamic imbalance between the accumulation kinetics of two proteins, PER and TIM, which form the complex that regulates gene expression. Chaos can also occur in this system under nonautonomous conditions, as a result of forcing a light-sensitive parameter by light-dark cycles. The picture emerging from the analysis of these various models is presented in Sec. VI where we compare the different routes leading from simple to complex oscillatory behavior in metabolic and genetic control networks.

II. INTERPLAY BETWEEN TWO ENDOGENOUS BIOCHEMICAL OSCILLATORY MECHANISMS

A. Two oscillatory enzyme reactions coupled in series

A simple two-variable model for periodic oscillations of the limit cycle type is provided by the case of a product-activated enzyme reaction [Fig. 1(A)]. Such a reaction forms the core of models proposed for glycolytic oscillations in yeast and muscle.[2] These oscillations originate from the positive feedback exerted on the enzyme phosphofructokinase (PFK) by ADP, a product of the enzyme reaction. A two-variable model taking into account the allosteric (i.e., cooperative) nature of the enzyme and the autocatalytic effect

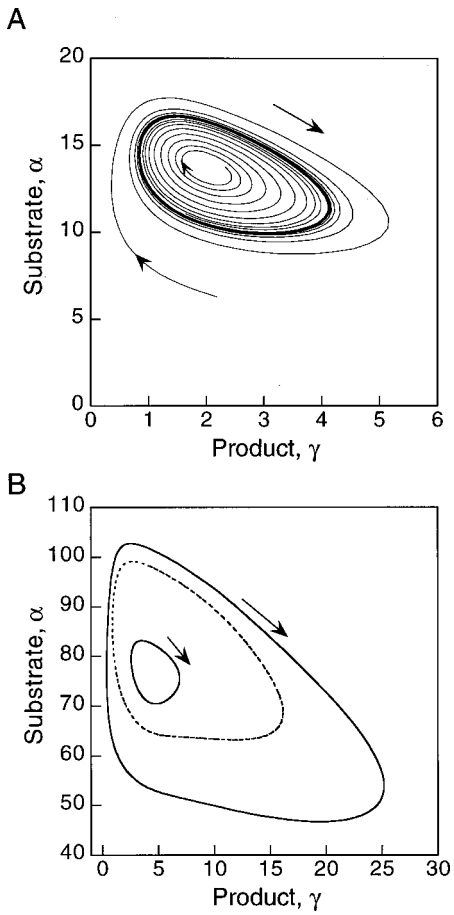

FIG. 2. (A) Limit cycle obtained in the model shown in Fig. 1(A). (B) Birhythmicity in the model of Fig. 1(B); depending on initial conditions, the system evolves toward either one of two stable limit cycles (solid curves) separated by an unstable limit cycle (dashed curve).

exerted by the product shows the occurrence of sustained oscillations: beyond a critical parameter value, the steady state admitted by the system becomes unstable and the system evolves toward a stable limit cycle [Fig. 2(A)] corresponding to simple periodic behavior. The model accounts for most experimental data, namely the existence of a domain of substrate injection rates producing sustained oscillations, bounded by two critical values of this control parameter, and the decrease in period observed when the substrate input rate increases.[2,4]

In the following, we will refer to this positive feedback regulation as an example of instability-generating mechanism. The core mechanism shown in Fig. 1(A) can be extended to allow for more complex modes of oscillatory behavior. If we keep the number of variables at two, and consider a nonlinear recycling of product into substrate [Fig. 1(B)], then a second stable limit cycle may appear in an appropriate range of parameter values.[5] Birhythmicity[3] occurs in these conditions: two stable limit cycles with different amplitudes and periods, separated by an unstable cycle, coexist for a given set of parameter values [Fig. 2(B)]. Depending on initial conditions, the system evolves toward either one of the stable limit cycles; the unstable limit cycle represents the separatrix between the basins of attraction of the two stable cycles. Such a phenomenon is the rhythmic counterpart of bistability in which two stable steady states coexist.

Yet a richer repertoire of dynamic behavior is obtained[3] when coupling in series two product-activated enzyme reactions [Fig. 1(C)]. The system now consists of three variables: substrate S is transformed by enzyme E_1 into product P_1 which itself serves as substrate for the transformation by enzyme E_2 into product P_2; this three-variable system is open to an influx of S, and to an efflux of P_2. The allosteric enzymes E_1 and E_2 are activated by their respective products, P_1 and P_2. The interplay between the two instability-generating mechanisms results in the occurrence in parameter space of two domains of instability; in each of these the steady state becomes unstable owing to one of the two positive feedback loops. It is when the two domains of instability overlap, due to the change in the value of some other parameter, that more complex modes of oscillatory behavior are encountered.[2,6]

As the rate constant measuring the efflux of end product progressively increases, the system evolves at first toward a limit cycle [Fig. 3(A)] associated with simple periodic oscillations. Then, after a sequence of period-doubling bifurcations it evolves toward a strange attractor [Fig. 3(B)] associ-

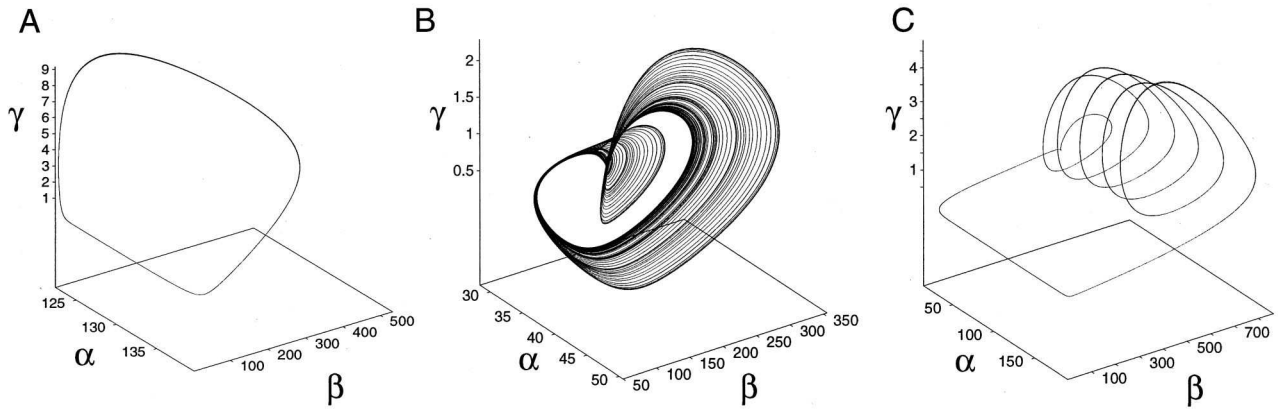

FIG. 3. Various phase plane trajectories obtained in the three-variable biochemical model schematized in Fig. 1(C): (A) limit cycle corresponding to simple periodic oscillations; (B) strange attractor corresponding to chaos; (C) folded limit cycle corresponding to complex periodic oscillations, i.e., bursting (see Ref. 2). α, β, γ denote the normalized concentrations of S, P_1, P_2 in Fig. 1(C).

ated with chaotic oscillations. Finally, oscillations become periodic again but acquire a complex form: several peaks per period are observed, giving the limit cycle a folded appearance [Fig. 3(C)]. The latter type of behavior corresponds to bursting, a mode of oscillatory behavior often observed in neurons and also exemplified by pancreatic β cells responsible for insulin secretion.[7,8] Hard excitation, i.e., the coexistence between a stable steady state and a stable limit cycle, and the coexistence between two (birhythmicity) and even three simultaneously stable limit cycles (trirhythmicity) can also be observed in this model.[2,9]

When establishing in parameter space the domains associated with these different types of dynamic behavior, the results indicate that in spite of the presence of two instability-generating mechanisms, the largest oscillatory domain remains that of simple periodic behavior. Thereafter, in order of decreasing frequency of occurrence, come hard excitation, complex periodic oscillations (bursting), birhythmicity, chaos, and trirhythmicity.[3]

B. Coupling two biochemical oscillators driving successive phases of the cell cycle

Another example of interplay between two oscillatory mechanisms is provided by the coupling of two oscillators through mutual inhibition. In a biochemical context, such a situation could underlie the periodic alternation of mitosis and DNA replication in the eukaryotic cell cycle. Each of these events is driven by an oscillatory enzyme cascade involving a cyclin and a cyclin-dependent protein kinase (cdk): mitosis is brought about by a rise in cdk1, while the onset of DNA replication is associated with an increase in cdk2.[10,11]

Detailed models have been proposed for the recurrent onset of DNA replication (S phase) and mitosis (M phase) in yeast and in somatic cells.[12,13] Here we will consider a simple model[14] consisting of two oscillatory enzyme cascades, each of which involves a cyclin (C), a kinase of the cdk type (M), as well as a protease (X) governing cyclin degradation. In each cascade, the accumulation of cyclin brings about the activation of the associated cdk through dephosphorylation; the rise in cdk results in the activation of the cyclin protease through phosphorylation. The increase in protease activity brings about a decline in cyclin and a new round of cdk activation may start as cyclin accumulation resumes. A negative feedback loop thus underlies cdk regulation, since cdk activation depends on cyclin and leads to cyclin degradation. The study of a three-variable system for the cell cycle in amphibian embryonic cells has shown that this negative feedback loop is capable of generating sustained oscillations of the limit cycle type, when the thresholds associated with phosphorylation-dephosphorylation kinetics are taken into account.[15]

To investigate how the two oscillators governing, respectively, the entry into mitosis and the onset of DNA replication may interact, we have considered the minimal model schematized in Fig. 4 where two cascades involving on one hand cdk1 (M_1) and its associated cyclin (C_1) and cyclin protease (X_1), and on the other hand the corresponding proteins (C_2 and X_2) associated with cdk2 (M_2) are coupled through mutual inhibition. The reason why we focus on the

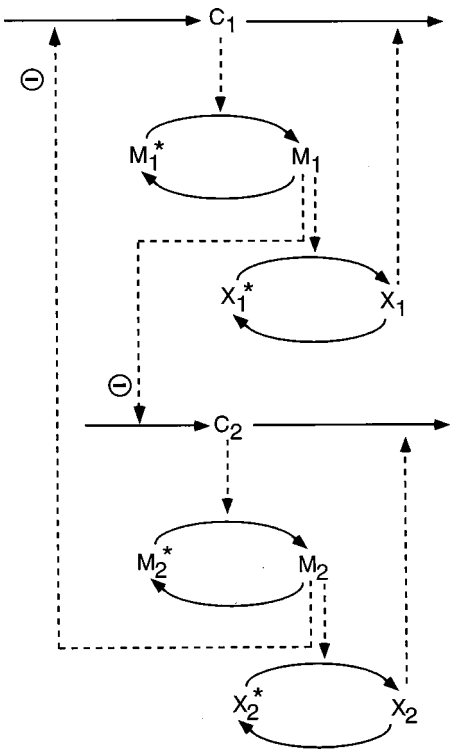

FIG. 4. Scheme of the minimal model of two biochemical oscillators controlling successive phases of the cell cycle (see Ref. 14). Each oscillator ($i=1,2$) represents an enzymatic cascade in which the accumulation of cyclin (C_i) triggers the activation of a cyclin-dependent kinase (M_i) which in turn activates a cyclin protease (X_i), thereby leading to cyclin degradation. The two oscillators are coupled through mutual inhibition.

case of inhibitory coupling stems from the observation that the S and M phases are ordered within the cell cycle, so that cells avoid dividing as long as their genetic material is not properly replicated. Two types of mutual inhibition have been investigated; in the first case, the active form of each cdk inhibits the synthesis of the cyclin associated with the other cdk (as schematized in Fig. 4), while in the second case (not shown) we assume that each cdk promotes the degradation of the cyclin associated with the other cdk. The two types of inhibitory coupling yield similar results.

The most common mode of dynamic behavior displayed by this system of two coupled oscillatory cascades is that of antiphase oscillations in which the corresponding variables of the two systems alternate.[14] Thus, the peak in C_1 is reached when C_2 is near its minimum, while C_1 goes down to its minimum as C_2 increases [Fig. 5(B)]. The same alternation is observed for the peaks in M_1 and M_2. The trajectory projected onto the (C_1-C_2) phase plane takes the form of an antisymmetric limit cycle [Fig. 5(A)]. Alternating oscillations of cdk1 and cdk2 represent the physiologically relevant situation which allows for the ordered succession of the M and S phases of the cell cycle. This mode of oscillatory behavior is obtained in conditions of strong mutual inhibition.

When mutual inhibition becomes weaker, oscillations can become chaotic [Fig. 5(D)] as the system evolves toward a strange attractor [Fig. 5(C)]. A coexistence between two

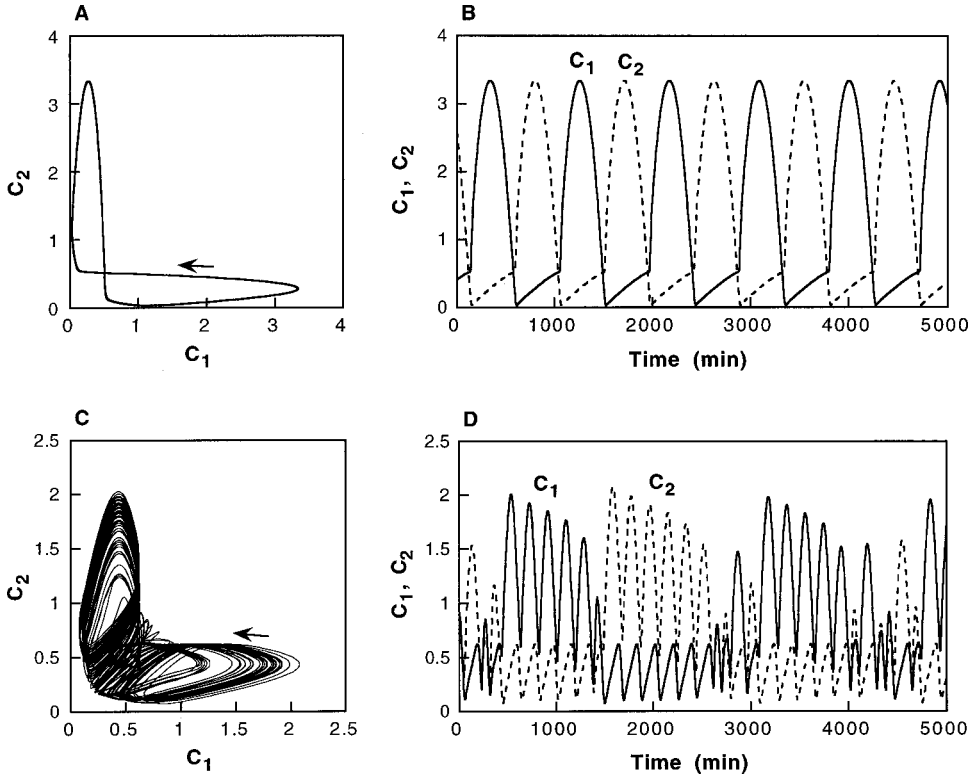

FIG. 5. Main types of dynamic behavior observed in the model schematized in Fig. 4 (see Ref. 14). (A) Projection of the limit cycle associated with antiphase oscillations shown in (B); (C) strange attractor corresponding to chaotic oscillations shown in (D).

stable limit cycles or between two stable strange attractors can also occur in such conditions.[14]

Much as in the case of two oscillatory enzymes coupled in series, the coupling of the two oscillators through mutual inhibition clearly results in the enrichment of the repertoire of oscillatory behavior, allowing for chaotic behavior and birhythmicity, in addition to simple periodic oscillations.

III. SELF-MODULATION OF AUTONOMOUS Ca^{2+} OSCILLATIONS

Over the last 15 years oscillations in intracellular Ca^{2+} have become a major example of oscillatory behavior at the cellular level.[16] These oscillations are observed in a large variety of cell types, with periods ranging from seconds to minutes; they occur either spontaneously or when a cell is stimulated by a hormone or a neurotransmitter. Intracellular waves of Ca^{2+} often accompany the oscillations; waves of Ca^{2+} may also propagate from cell to cell, as observed in endothelial and liver tissue.[17]

The mechanism of Ca^{2+} oscillations—and that of associated waves—rests on the regulation of Ca^{2+} levels within the cell. Upon binding to a specific membrane receptor, the external stimulus triggers the synthesis of an intracellular messenger, inositol 1,4,5-triphosphate ($InsP_3$) which binds to the $InsP_3$ receptor which behaves as a Ca^{2+} channel and is located in the membrane of intracellular Ca^{2+} stores. Binding of $InsP_3$ to the receptor elicits the release of Ca^{2+} from these stores into the cytosol. The release of Ca^{2+} is also activated by cytosolic Ca^{2+} in a process known as Ca^{2+}-induced Ca^{2+} release (CICR). This self-activation process represents a key element in the instability mechanism that generates repetitive Ca^{2+} spiking.[18,19]

Two forms of CICR are known. When Ca^{2+} transport is mediated by the ryanodine receptor, as occurs in the sarcoplasmic reticulum in muscle and cardiac cells, CICR can operate in the absence of $InsP_3$. When Ca^{2+} transport is mediated by the $InsP_3$ receptor, as occurs in the endoplasmic reticulum, Ca^{2+} and $InsP_3$ behave as coagonists in triggering Ca^{2+} release from intracellular stores. The latter process may be viewed as an $InsP_3$-sensitive CICR. From a dynamical point of view, the two modes of CICR are equivalent.[19] Both are capable of producing sustained oscillations of cytosolic Ca^{2+} and will not be further distinguished later.

A variety of models for Ca^{2+} oscillations have by now been proposed (see Ref. 20 for a recent review). Differing by the degree of detail with which the dynamics and control of the $InsP_3$ receptor are treated, most of these models are based on CICR as the main instability-generating mechanism. A typical model based on CICR (Fig. 6) accounts for the occurrence of repetitive Ca^{2+} spiking [Fig. 7(A)] in the form of limit cycle oscillations [Fig. 7(B)]. Such simple periodic oscillations occur in a domain bounded by two critical values of the stimulation level which represents the main control parameter in the experiments. Below the lower critical value of the external stimulation, the system reaches a stable steady state corresponding to a constant low level of cytosolic Ca^{2+}. Above the higher critical value, a constant high level of cytosolic Ca^{2+} is maintained. Thus, a window of oscillatory behavior separating a low and a high Ca^{2+} steady state is obtained as a function of the stimulation intensity, both in model and experiment. The model also accounts for the observation that the frequency of the oscillation increases as the stimulation rises.[18,19,21]

In some cells, Ca^{2+} oscillations acquire a complex ap-

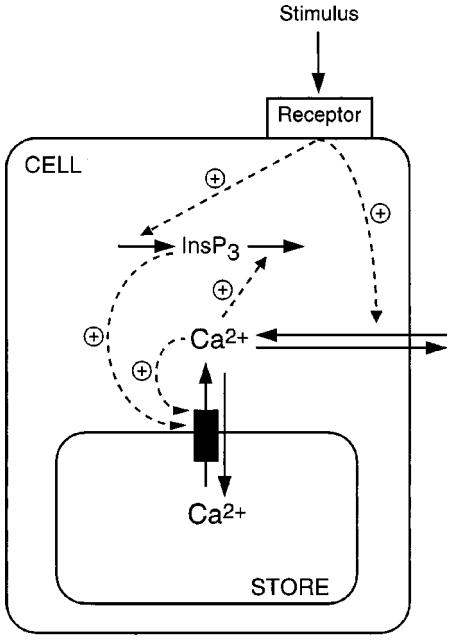

FIG. 6. Model for simple and complex intracellular Ca^{2+} oscillations based on Ca^{2+}-induced Ca^{2+} release (see Ref. 26). The model incorporates the triggering of $InsP_3$ synthesis following the binding of the external signal to a specific receptor, the activation of Ca^{2+} release from intracellular stores by Ca^{2+} and $InsP_3$, as well as the activation by Ca^{2+} of $InsP_3$ metabolic transformation by the enzyme 3-kinase.

pearance. Sometimes they resemble bursting, with a series of Ca^{2+} spikes of varying magnitude separated from another group of spikes by a silent interval. This oscillatory behavior may be periodic or chaotic. The question arises as to how models may account for the transition from simple to complex Ca^{2+} oscillations, including chaos. A variety of models for Ca^{2+} oscillations are capable of displaying either simple periodic behavior or chaos. These models often rely on the interplay between two instability-generating mechanisms, as discussed earlier in Sec. II. For example, CICR may provide a first oscillatory mechanism while the second relies on the activation of $InsP_3$ synthesis by Ca^{2+}.[22] The latter regulation, observed in some cell types, also represents a form of positive feedback and was shown to be capable of generating sustained Ca^{2+} oscillations.[23] A different model admitting complex Ca^{2+} oscillations is based on experiments performed in hepatocytes and involves the negative regulation of the α subunit of a G protein by phospholipase C and by cytosolic Ca^{2+}.[24]

Another possible source of complex oscillatory behavior has been uncovered in the study of models for Ca^{2+} signaling incorporating the dynamics of $InsP_3$ synthesis and degradation.[25,26] The mechanism relies on self-modulation by the oscillatory system of one of its main control parameters. As shown in Fig. 6, $InsP_3$ is synthesized in response to external stimulation and, together with cytosolic Ca^{2+}, triggers the release of Ca^{2+} from intracellular stores. A further coupling between Ca^{2+} and $InsP_3$ exists, because cytosolic Ca^{2+} activates the enzyme 3-kinase which metabolizes $InsP_3$ into $InsP_4$. This additional regulation can have profound effects on the dynamics of intracellular Ca^{2+}. Indeed, through the control of the 3-kinase, Ca^{2+} oscillations modulate the level of $InsP_3$ which itself controls Ca^{2+} oscillations. In this model, which contains three variables—namely the cytosolic concentration of $InsP_3$ and the concentration of Ca^{2+} in the cytosol and in the intracellular stores—simple periodic oscillations of Ca^{2+} in the form of spiking [Fig. 7(A)] corre-

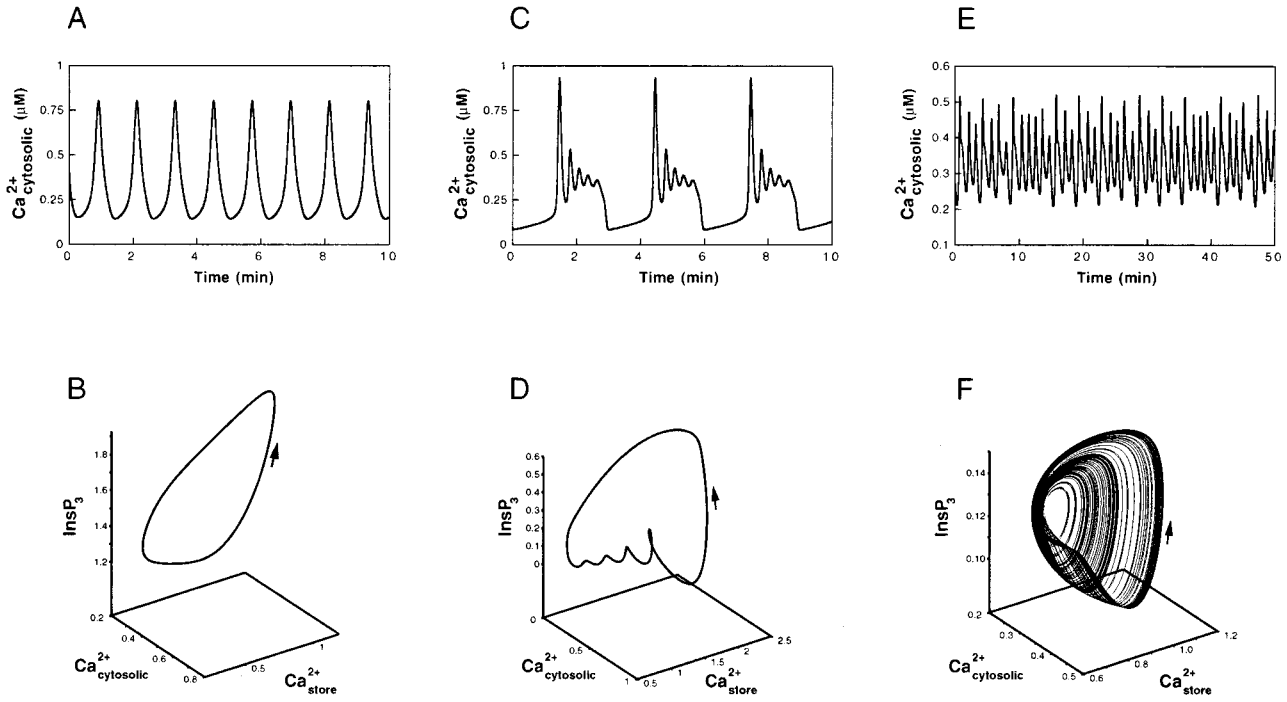

FIG. 7. Simple (A) and complex (C) periodic oscillations and chaos (E) obtained in three-variable model for Ca^{2+} signaling schematized in Fig. 6 (see Ref. 26). The phase space trajectories corresponding to these time evolutions are shown in panels (B), (D) and (F), respectively.

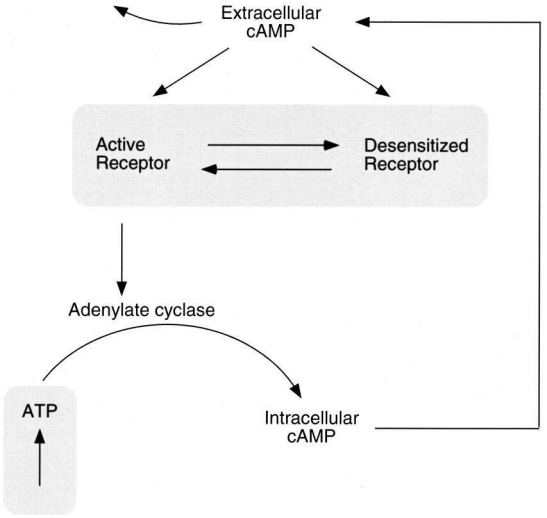

FIG. 8. Regulatory mechanism underlying simple and complex cAMP oscillations in *Dictyostelium* cells (see Ref. 2). Self-amplification results from the activation of the cAMP-synthesizing enzyme adenylate cyclase following binding of extracellular cAMP to the cAMP receptor. This positive feedback loop is counteracted by two limiting processes (shown in gray): reversible desensitization of the cAMP receptor and consumption of the substrate ATP; a third limiting factor is the hydrolysis of cAMP by phosphodiesterase.

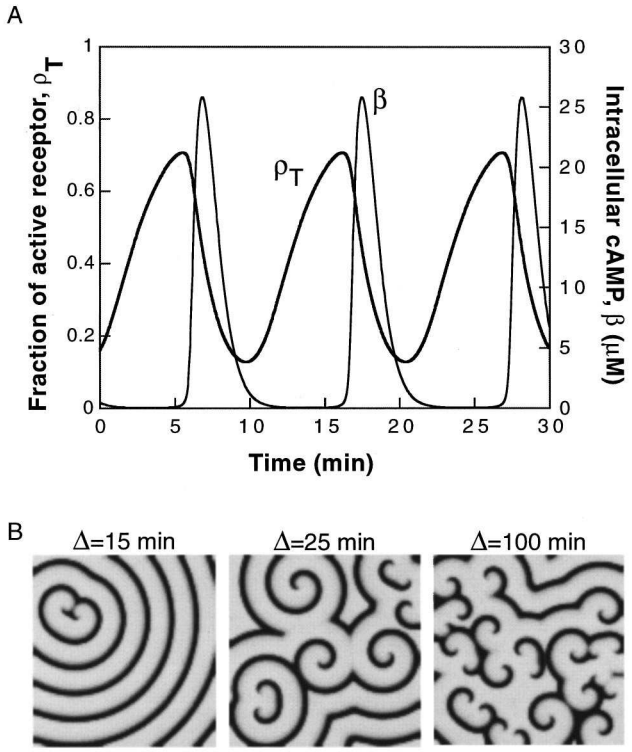

FIG. 9. (A) Sustained oscillations obtained in the model for cAMP signaling based on receptor desensitization schematized in Fig. 8. Shown are the time evolution of the normalized concentration of intracellular cAMP (β) and the fraction of active receptor (ρ_T). (B) When diffusion of extracellular cAMP is incorporated into the model for cAMP signaling based on receptor desensitization, waves of cAMP are observed, which are (from left to right) concentric or take the form of a few large spirals or numerous smaller spirals, as the value of the degree of desynchronization Δ progressively increases (see Ref. 36).

sponding to the evolution toward a limit cycle [Fig. 7(B)] remain the most common type of oscillatory behavior.

As a result of self-modulation of the $InsP_3$ level by Ca^{2+}, more complex oscillatory phenomena are also observed. Thus, Ca^{2+} oscillations of the bursting type, similar to the experimentally observed complex Ca^{2+} oscillations can occur [Fig. 7(C)]; these oscillations correspond to a folded limit cycle in the three-variable phase space [Fig. 7(D)]. Chaos also occurs in this model [Fig. 7(E)] through a sequence of period-doubling bifurcations; the phase space trajectory then takes the form of a strange attractor [Fig. 7(F)].

IV. PERIODIC OSCILLATIONS AND CHAOS IN cAMP SIGNALING IN *DICTYOSTELIUM*

Intercellular communication by pulsatile cAMP signals in *Dictyostelium discoideum* amoebae represents one of the best examples of pulsatile signaling in intercellular communication and of spatiotemporal organization at the cellular level.[2] These amoebae aggregate after starvation by a chemotactic response to cAMP signals emitted by cells behaving as aggregation centers.[27] The aggregation possesses a wavelike nature, because of the pulsatile release of cAMP signals by the centers.[27,28] Experiments in cell suspensions have confirmed that cAMP in *D. discoideum* is synthesized in an oscillatory manner, with a period of the order of 10 min.[29]

The mechanism that underlies the periodic synthesis of cAMP in *D. discoideum* again involves a positive feedback loop: extracellular cAMP binds to a cell surface receptor and thereby, via the action of G proteins, activates adenylate cyclase, the enzyme that transforms ATP into cAMP (Fig. 8). Intracellular cAMP thus synthesized is transported into the extracellular medium where it becomes available for binding to its receptor. As indicated by models for cAMP signaling in *D. discoideum*, the self-amplification resulting from this positive feedback loop is at the core of the instability mechanism leading to oscillations.[2]

To avoid a biochemical explosion, self-amplification must be limited by a second biochemical process which here can take several forms. The first limiting factor is the hydrolysis of intracellular and extracellular cAMP by phosphodiesterase. A second factor is that of substrate consumption; however, the level of ATP was found to vary only slightly in the course of cAMP oscillations. A third limiting process is that of receptor desensitization: upon binding cAMP, the cAMP receptor becomes phosphorylated; dephosphorylation occurs as soon as the ligand is removed.[30] Several lines of evidence suggest that the phosphorylated receptor is desensitized, i.e., cannot activate adenylate cyclase as well as the dephosphorylated receptor form. As in sensory systems, receptor desensitization underlies cellular adaptation to constant stimuli, even though it appears that bypass mechanisms allow adaptation in the absence of receptor phosphorylation.[31]

A model for cAMP signaling based on receptor desensitization accounts for autonomous oscillations of cAMP [Fig. 9(A)] and relay (i.e., excitability) of suprathreshold cAMP pulses.[32] This model also explains why pulsatile signals of

cAMP are more efficient than constant stimulation in triggering a cellular response; constant stimulation indeed leads to receptor desensitization. The model indicates that there exists an optimal pattern of pulsatile signaling which strongly depends on the kinetics of desensitization and resensitization of the cAMP receptor.[2,33]

Besides periodic oscillations in cAMP, the model also predicts the possibility of bursting and of aperiodic synthesis of cAMP signals.[32,34] While simple periodic oscillations can occur when the level of ATP remains constant, complex oscillations were found only when the level of ATP was allowed to vary, even though the range of variation of ATP remained reduced. The mechanism leading from simple periodic behavior to bursting and chaos relies here again on the interplay between two endogenous oscillatory mechanisms. These two mechanisms share the same positive feedback loop in cAMP synthesis but involve distinct limiting processes: the first relies on receptor desensitization, while the second relies on substrate consumption (Fig. 8). It is when the two limiting processes acquire comparable importance, i.e., when the two oscillatory mechanisms are simultaneously active, that oscillations acquire a chaotic or bursting nature.

The role of cAMP signals is to govern intercellular communication in *Dictyostelium discoideum* cells. The intercellular nature of this rhythmic process raises the question of the efficacy of periodic versus chaotic or random signaling. The analysis of the model based on receptor desensitization showed that there exists an optimal pattern of pulsatile signaling that maximizes the capability of cells to synthesize cAMP in response to an extracellular cAMP pulse.[33] This pattern is characterized by a pair of optimal values for the duration of a pulse and the interval between successive pulses. When comparing the response of the signaling system to various patterns of pulsatile cAMP stimulation, this optimal periodic pattern proves more efficient than pulses delivered in a chaotic or random manner.

The observation of chaotic behavior in the model for cAMP signaling in *Dictyostelium* raises the question of whether chaos has been observed in the course of slime mold aggregation. The *D. discoideum* mutant *Fr17* could provide such an example, as its aggregation was shown to proceed in an aperiodic manner. The study of the model for cAMP signaling shows, however, that chaos might be difficult to observe in cell suspensions which are often used to demonstrate cAMP oscillations. It suffices indeed that a few percent of periodic cells are present in a suspension containing some 95% of cells behaving chaotically for the whole cell population to become periodic,[35] because of the synchronizing effect exerted by extracellular cAMP. The addition of a small percentage of periodic cells can therefore suppress chaos in a population of initially chaotic cells.

When incorporating diffusion of extracellular cAMP into the model based on receptor desensitization, waves of cAMP can be obtained. These waves are concentric if a center emitting autonomously pulses of cAMP is placed in the middle of a field of excitable cells which relay the signal emitted periodically by the center. The question arises as to the origin of spiral waves of cAMP which are often observed in the course of *D. discoideum* aggregation. The model indicates[36,37] that spiral waves may originate naturally when taking into account the changes in dynamic properties due to the progressive increase in adenylate cyclase and phosphodiesterase during the hours after starvation, and the desynchronization of cells with respect to such biochemical changes. Depending on the value of a parameter denoted Δ which characterizes the degree of this desynchronization, waves are either concentric (when Δ remains reduced), or take the form of a small number of large spirals which transform into a large number of minute spirals as the value of Δ increases [Fig. 9(B)].

V. PERIODIC BEHAVIOR AND CHAOS IN A MODEL FOR CIRCADIAN RHYTHMS BASED ON GENETIC REGULATION

A. Autonomous chaos resulting from dynamic imbalance between two proteins forming a regulatory complex

In the earlier discussion of scenarios leading from simple to complex oscillations, a recurrent theme has been that the interplay between two instability-generating mechanisms often underlies such transitions. Yet another example related to such a scenario is provided by a model for circadian oscillations. The model, schematized in Fig. 10(A), was proposed[38,39] to account for the 24 h periodicity observed in the levels of the PER and TIM proteins in *Drosophila*. Experiments carried out over the last decade have shown[40–42] that the mechanism underlying these circadian oscillations rests on a transcriptional regulatory loop [see Fig. 10(A)]; the *per* and *tim* genes are transcribed in the nucleus; the messenger RNA molecules thus synthesized go into the cytoplasm where they are translated into the PER and TIM proteins. The latter are phosphorylated and form a complex which migrates into the nucleus where it inhibits the expression of the *per* and *tim* genes. This negative transcriptional feedback loop[43] lies at the core of the mechanism producing sustained oscillations.

The model based on this negative feedback regulation is described by a system of ten nonlinear ordinary differential equations[38,44] which govern the time evolution of the various protein and mRNA species. Light controls circadian oscillations in *Drosophila* by inducing rapid degradation of the TIM protein. This model accounts for a variety of experimental observations pertaining, for example, to the occurrence of circadian oscillations of PER and TIM in continuous darkness, the entrainment of the oscillations by light-dark cycles, and the phase shifts induced by light pulses.[38,39]

In addition to simple periodic oscillations, complex oscillatory phenomena, including chaos [Figs. 11(A) and 11(B)] and birhythmicity can also occur in this model,[38,44,45] even though it contains a single regulatory loop, namely the negative feedback exerted by the PER–TIM complex on the expression of the *per* and *tim* genes. Complex oscillatory phenomena originate here from the fact that two distinct branches lead to the formation of the PER–TIM regulatory complex. The two branches involve, respectively, the synthesis and degradation of the *per* and *tim* mRNAs and of the PER and TIM proteins. Complex oscillatory behavior arises

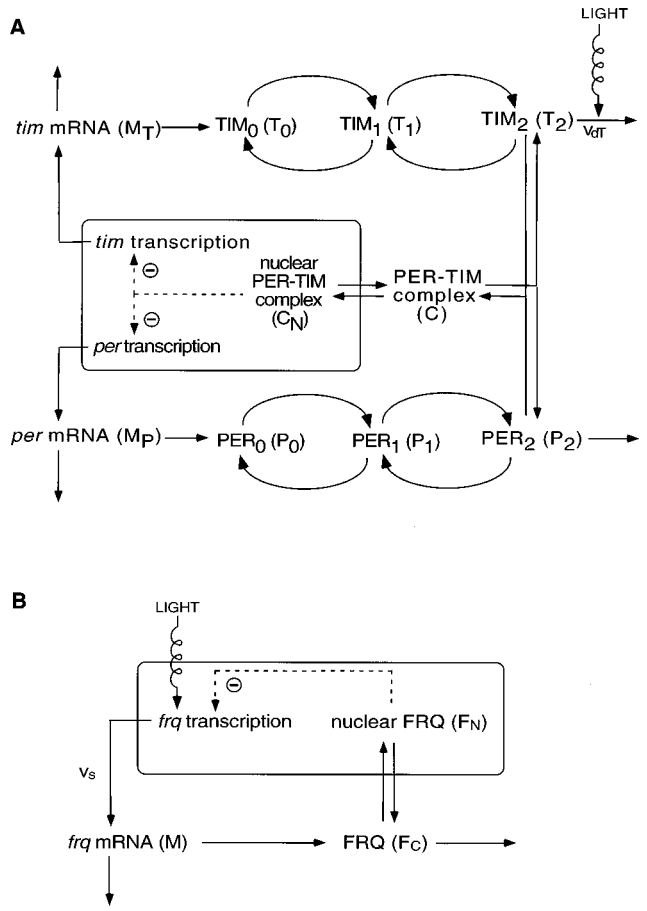

FIG. 10. (A) Model for circadian rhythms in *Drosophila* based on transcriptional regulation (see Ref. 38). The model incorporates transcription of the *per* and *tim* genes, translation of the *per* and *tim* mRNAs into the proteins PER and TIM (P_0, T_0), reversible phosphorylation of the two proteins (modification of P_0 and T_0 into the forms P_1, T_1, and P_2, T_2, successively), formation of a complex between the PER and TIM proteins and transport of this complex into the nucleus, where it represses the transcription of the *per* and *tim* genes. Light controls the circadian rhythm by triggering TIM degradation. (B) Model for circadian rhythms in *Neurospora* based on transcriptional regulation (see Ref. 47). This minimum model is based on the negative control exerted by the protein FRQ on the expression of its gene *frq*; light acts by triggering the transcription of this gene.

when these two branches are not characterized by the same kinetic parameters (that dissimilarities exist is indeed suggested by the differences observed in the levels of PER and TIM). Only simple periodic oscillations are observed in the model when these two branches are characterized by similar kinetic parameter values.

B. Nonautonomous chaos resulting from the periodic forcing of a circadian oscillator by light-dark cycles

All cases discussed so far pertain to the autonomous occurrence of simple or complex oscillatory behavior, in the absence of any periodic forcing. We wish to address here the case of nonautonomous chaos in a model for circadian oscillations, for two main reasons. First, forcing sustained oscillations by a periodic input is a well known way to produce chaos, as demonstrated by a variety of experimental and theoretical studies in chemical, physical and biological systems.[46] Second, while the existence of a periodic input in biological systems is often artificial in a physiological contact, this is not the case for circadian rhythms. All living organisms indeed operate in a periodically varying environment, characterized by the 24 h periodic alternation of day and night. It is thus necessary to ask whether the existence of the natural light-dark cycles can bring circadian oscillations to acquire a chaotic nature, even though from a physiological point of view this would jeopardize the main function of these rhythms, which is to allow the organisms to adapt to the periodicity of their environment.

We have investigated the effect of periodic forcing in a minimal three-variable model proposed for circadian oscillations of the FRQ protein in *Neurospora*.[44] The mechanism underlying these oscillations is also based on a negative transcriptional regulatory loop.[41] Here [see Fig. 10(B)], the nuclear form of the protein FRQ exerts a negative feedback on the transcription of its gene *frq*, and light acts by promoting the expression of the *frq* gene. The influence of the external light-dark (LD) cycle is taken into account by considering that the maximum rate of *frq* transcription varies in a periodic manner, with a periodicity dictated by that of the forcing LD cycle.[47,48] Chaos can readily be obtained in such nonautonomous conditions [Figs. 11(C) and 11(D)], although entrainment to the external input remains more common—this fits with the expected physiological role of the external LD cycle which should entrain the organism to oscillate with a 24 h period.

In assessing the effect of forcing circadian oscillations by the external LD cycle, we have investigated the influence of the external period as well as the wave form of the periodic variation in the light-sensitive parameter.[48] Thus, we have considered wave forms ranging from square-wave [Fig. 12(A), curve (a)] to sinusoidal [Fig. 12(A), curve (c)], and intermediate situations in which over a period τ, the parameter remains at a high value during time τ_1 and at a minimum during time τ_2, while τ_{12} and τ_{21} denote the durations of the sinusoidal transitions between these extreme values [Fig. 12(A), curve (b)]. In the case of a square wave, $\tau_{12}=\tau_{21}=0$, while in the case of a purely sinusoidal variation, $\tau_1=\tau_2=0$. Shown in Fig. 12(B) are the results of computer simulations indicating the effect of the wave form of an imposed 24 h periodic variation in the light-sensitive parameter which, in this case, represents the maximum rate of gene expression, denoted $\nu_{s\,max}$. The wave form is progressively varied from square-wave to sinusoidal by increasing the values of the transition times $\tau_{12}=\tau_{21}$. For each of these values the dynamic behavior of the model was determined as a function of the amplitude of the periodic variation in the parameter. In the case considered, the autonomous period of oscillations (corresponding to sustained oscillations observed in constant darkness in *Neurospora*) is close to 21.5 h.

The first column in Fig. 12(B) pertains to the case of a square-wave parameter variation. As the amplitude—i.e., the value of $\nu_{s\,max}$—progressively increases, quasiperiodic oscillations (QP) are obtained, then entrainment (E), followed by a sequence of period-doubling bifurcations (PD) leading to chaos. When the wave form gradually changes from square-wave to sinusoidal [left to right columns in Fig. 12(B)], the

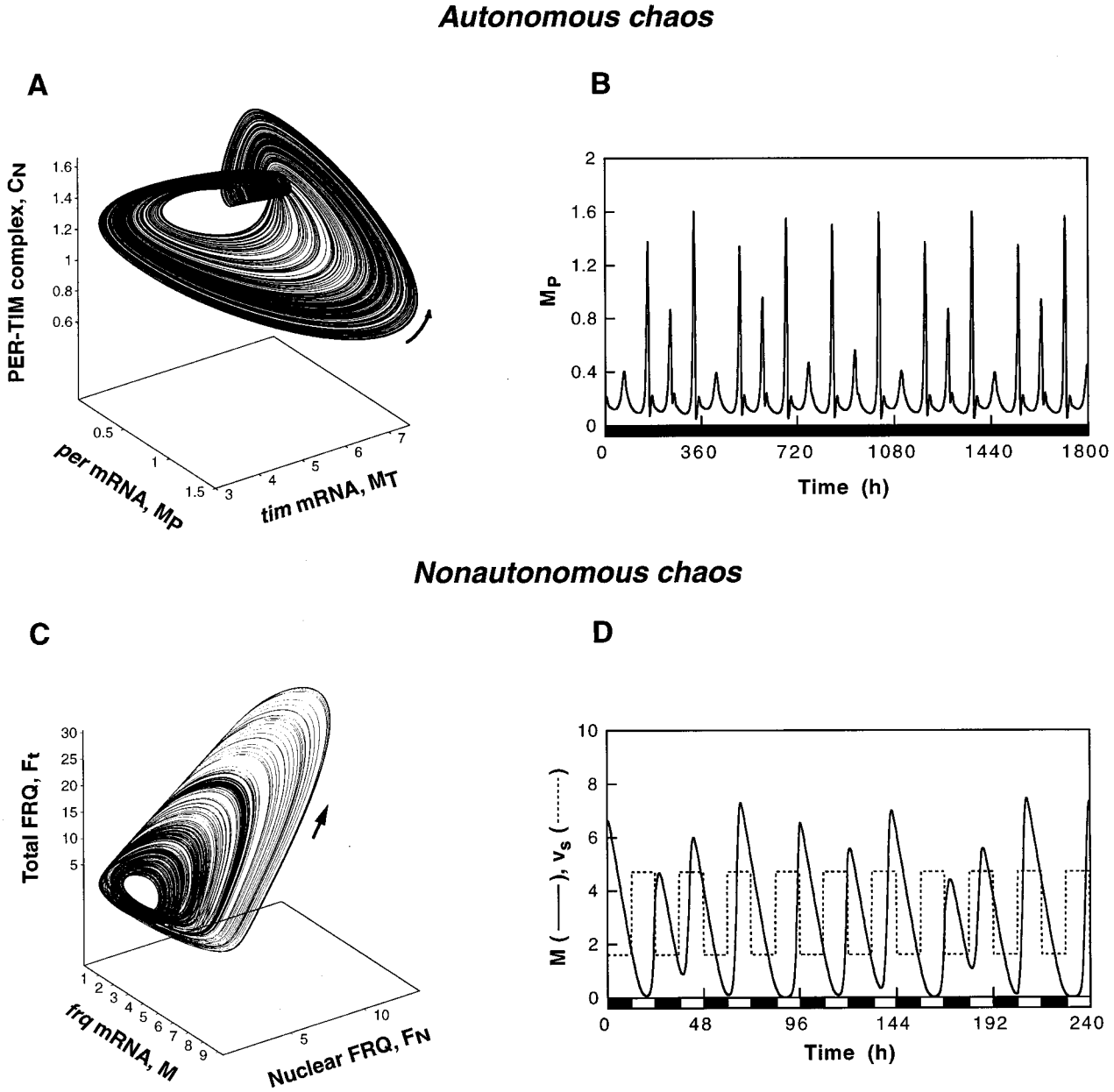

FIG. 11. (A) Strange attractor corresponding to autonomous chaotic oscillations (B) obtained in the ten-variable model for circadian oscillations in *Drosophila*, schematized in Fig. 10(A), in conditions corresponding to continuous darkness (see Ref. 45). (C) Strange attractor corresponding to nonautonomous chaos (D) obtained in the model for circadian oscillations in *Neurospora* schematized in Fig. 10(B), in conditions where the three-variable system is subjected to periodic forcing by a light-dark cycle (see Ref. 47). The effect of this periodic forcing takes here the form of a square-wave variation in parameter ν_s which measures the maximum rate of *frq* transcription.

domain of chaos shrinks and eventually vanishes while the domain of entrainment increases. These results indicate the importance of the wave form of the periodic input in eliciting the transition from simple periodic oscillations (corresponding to entrainment) to complex oscillations, including chaos.

VI. DISCUSSION

The purpose of this paper was to briefly review, by means of a number of selected examples, mechanisms capable of producing simple or complex modes of oscillatory behavior in metabolic and genetic control networks. The models considered were originally proposed to account for simple periodic oscillations observed experimentally at the cellular level in a variety of biological systems. In a second stage, these models were modified to allow for complex oscillatory phenomena such as bursting, birhythmicity, or chaos. We considered successively (1) a model based on enzyme regulation, proposed for glycolytic oscillations and a minimal model of biochemical oscillators controlling successive phases of the cell cycle, (2) a model for intracellular Ca^{2+} oscillations based on transport regulation, (3) a model for oscillations of cAMP based on receptor desensitization in *Dictyostelium* cells, and (4) a model based on transcriptional regulation for circadian rhythms in *Drosophila*.

In these various examples of metabolic or genetic oscillations, the molecular mechanism of periodic behavior rests

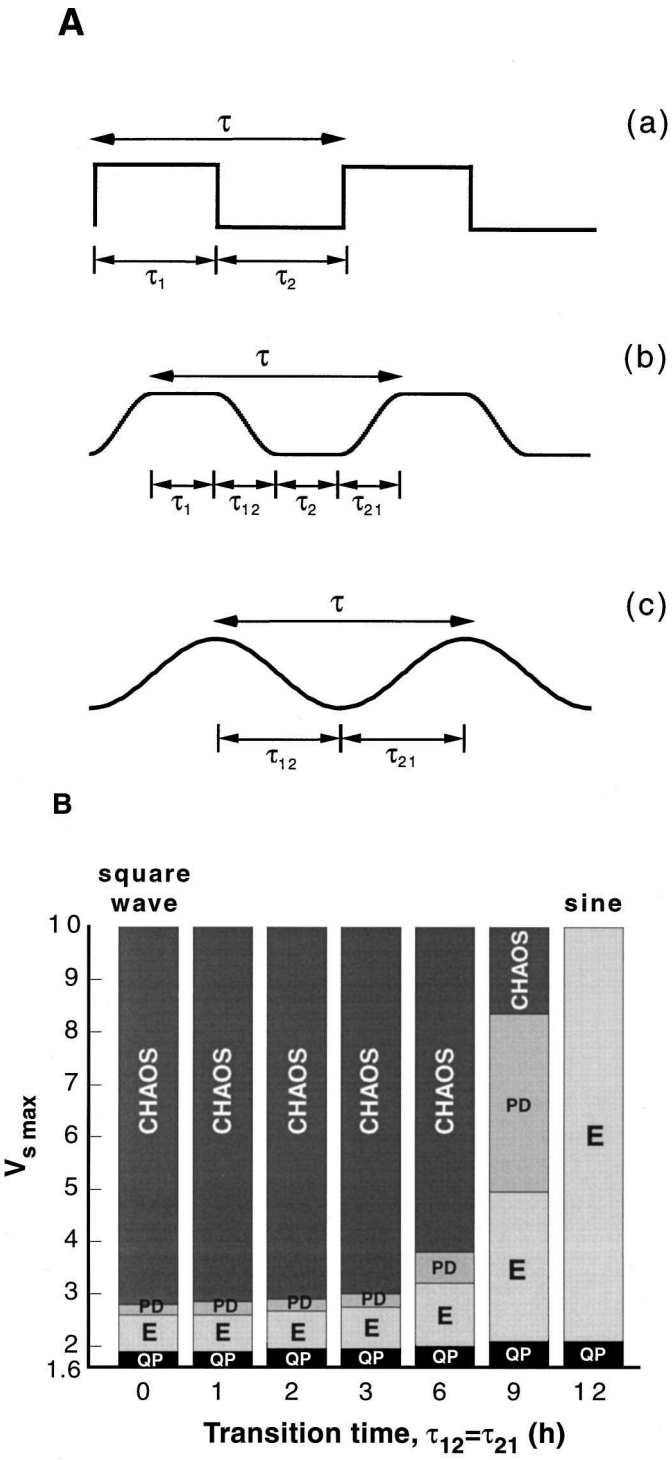

FIG. 12. (A) Types of wave form considered for the periodic forcing of circadian oscillations by a light-dark cycle (see Ref. 48). The wave form ranges from square-wave [curve (a)] to sinusoidal [curve (c)]; curve (b) represents an intermediate situation. (B) Domains of entrainment (E), quasi-periodic oscillations (QP), period-doubling (PD), and chaos obtained in the model of Fig. 10(B) when parameter ν_s is varied periodically in a manner ranging from square-wave to sinusoidal. The domains were determined for different values of the amplitude of periodic forcing, measured by the maximum value of parameter ν_s (see Ref. 48).

on the existence of nonlinear feedback regulatory processes. The regulatory feedback can be of negative nature, as in the minimal model for cdk oscillations in the cell cycle, or in the model for transcriptional regulation underlying circadian oscillations. Alternatively, the feedback can be of a positive nature as illustrated by the cases of glycolytic oscillations, cAMP oscillations in *Dictyostelium* cells, and Ca^{2+} oscillations. The feedback regulation that underlies periodic behavior can be exerted at different levels and occurs in a variety of ways: control of enzyme activity by allosteric regulation (as illustrated by the activation of PFK in glycolytic oscillations) or by phosphorylation-dephosphorylation (control of cdks in cell cycle oscillations); control of enzyme activity and of receptor function (cAMP oscillations in *Dictyostelium*); regulation of transport processes (role of CICR in Ca^{2+} oscillations); and regulation of gene expression (control of gene transcription underlying circadian rhythms).

That formally similar feedback processes can underlie oscillations in widely different contexts is further illustrated by the case of recurrent cyclic inhibition which underlies the ordered, rhythmic operation of a number of neural networks (a simple form of this cyclic organization of negative feedback loops is provided by a neuron A inhibiting neuron B which inhibits neuron C which, in turn, inhibits A).[49,50] A genetic regulatory network possessing a similar feedback structure based on three repressors coupled through cyclic inhibition (A represses B which represses C repressing A) has recently been constructed in bacteria and shown to display sustained oscillations.[51]

After examining the models proposed for a variety of biochemical and cellular rhythms, we focused on the different scenarios leading from simple to complex oscillatory behavior. In order to reach some global conclusions on the basis of the results obtained with these models, it is useful to classify the various mechanisms responsible for the transition from periodic oscillations to bursting, birhythmicity or chaos. Two general classes of scenario can be identified, both of which can be implemented in a variety of ways. The first scenario relies on the interplay between two instability-generating mechanisms, i.e., two mechanisms capable of producing endogenous oscillatory behavior. The second involves the self-modulation by an oscillatory system of a parameter controlling the oscillations.

The coupling between two mechanisms capable of producing endogenous oscillations can be overt or subtle. To the first class belong the model examined in Sec. II A where two autocatalytic enzyme reactions are coupled in series, and the model presented in Sec. II B where two biochemical oscillators controlling distinct phases of the cell cycle are coupled through mutual inhibition. In these models, the number of instability-generating mechanisms, i.e., two, is equal to the number of regulatory feedback loops capable of generating autonomous oscillations. These regulatory loops correspond either to positive feedback, as in the model for glycolytic oscillations which rely on the activation of an allosteric enzyme by a reaction product (Sec. II A), or to negative feedback, as in the cascade model based on cdk-induced cyclin degradation (Sec. II B).

In other models, the coupling between two oscillatory mechanisms is more subtle, as only a single instability-generating feedback loop can be recognized, despite the existence of two distinct oscillatory mechanisms. The reason is that the two mechanisms share the same feedback loop, but

differ in some other respect. Thus, in the model for cAMP signaling in *Dictyostelium* (Sec. IV), two oscillatory mechanisms share the positive feedback exerted by extracellular cAMP on the cAMP-synthesizing enzyme adenylate cyclase via binding to the cAMP receptor located on the cell membrane; the factor limiting this self-amplification is either substrate consumption or receptor desensitization. These two limiting factors coexist, and complex oscillatory phenomena occur when they acquire similar importance in controlling the dynamics of the cAMP signaling system. Interestingly, it suffices that the substrate ATP is allowed to vary, even slightly, for chaotic or bursting oscillations to occur. Even in these conditions, however, periodic behavior remains the most common type of dynamic behavior (see later).

A second example of coupling between two oscillatory mechanisms in the presence of a single regulatory feedback loop is provided by the model for circadian rhythms in *Drosophila* (Sec. V). In this case the coupling between two endogenous oscillatory mechanisms takes yet another form. The single instability-generating regulatory loop involves the negative feedback exerted by the PER–TIM complex on the expression of the *per* and *tim* genes. The key fact is that two distinct proteins assemble to form this regulatory complex. Complex oscillatory phenomena originate here from a dynamic imbalance between the relative rates of accumulation of each of these two proteins (and/or their mRNAs). Such an imbalance results from the existence of differences in the kinetics of synthesis and degradation of the PER and TIM proteins and their mRNAs. Besides birhythmicity and chaos, the PER–TIM model can also show for certain parameter values a coexistence between a stable steady state and stable oscillations. This phenomenon of hard excitation may play a role in the mechanism of long-term suppression of circadian rhythms by a single light pulse.[52]

The earlier discussion shows that the interplay between two endogenous oscillatory mechanisms can serve as a common source of complex oscillatory behavior in regulated biological systems. This conclusion can be related to the observation made in numerous experimental and theoretical studies that forcing an oscillatory system by a periodic input can readily produce bursting or chaos (see, for example, the study of the forcing of glycolytic oscillations by a periodic substrate input).[53] In the latter case, however, chaos is nonautonomous, in contrast to the results on autonomous chaos shown or mentioned in Secs. II–IV and V A. The link between the two situations is clear: in one case, a first oscillator provides an external periodic input that drives a second oscillator (which is based on endogenous regulation and can oscillate even in the presence of a constant input), whereas in the autonomous case, the two coupled oscillators are both endogenous and no periodic forcing is present in the environment.

Although we primarily focused on the conditions leading to simple or complex autonomous oscillations, we also considered the effect of forcing circadian oscillations by a periodic input. These oscillations are indeed unique by the property of being sensitive to light and naturally subjected to the periodic alternation of day and night. The study of the influence of forcing a circadian oscillator by light-dark cycles confirms that entrainment or chaos can occur in these conditions. Of particular interest is the result that, besides the amplitude and period of the periodic input, its wave form also markedly affects the dynamic behavior of the circadian oscillator. As shown in Fig. 12(B) the domain of entrainment indeed increases in parameter space at the expense of the domain of chaos as the wave form changes from square-wave to sinusoidal.

Another class of mechanism identified for the transition from simple to complex oscillatory behavior rests on the self-modulation of an autonomous oscillatory system. This source of complex oscillatory behavior could be widespread, even though we encountered it so far only in a model for intracellular Ca^{2+} oscillations (Sec. III). There, a stimulation-induced increase in the intracellular messenger $InsP_3$ triggers the release of Ca^{2+} from intracellular stores, in a process known as Ca^{2+}-induced Ca^{2+} release. This self-amplified process lies at the core of the instability mechanisms that produces sustained oscillations in the form of intracellular Ca^{2+} spiking. This model accounts well for simple periodic Ca^{2+} oscillations which are observed in a large variety of cell types, either spontaneously or after stimulation by a neurotransmitter or hormonal signal. Self-modulation of the oscillating system by its oscillatory activity occurs here because $InsP_3$ degradation is regulated by Ca^{2+}. Thus, the parameter that controls Ca^{2+} oscillations is itself modulated by the oscillating level of Ca^{2+}. Complex oscillatory phenomena, including bursting, birhythmicity and chaos, have been shown to arise from such a mechanism of self-modulation.[26]

Whether the self-modulation by an oscillating system of a control parameter represents a separate class of mechanism leading to complex oscillations is not clear cut. The distinction between the two classes of mechanisms is somewhat artificial, because self-modulation of a parameter controlling oscillations can also be viewed as resulting from the interplay between two instability-generating mechanisms. In the model of Fig. 6, one mechanism relies on the positive feedback provided by CICR, while the second mechanism would involve negative feedback on $InsP_3$. The latter messenger triggers the release of Ca^{2+} which activates the 3-kinase that degrades $InsP_3$. A characteristic of the self-modulation mechanism nevertheless remains that Ca^{2+} oscillations modulate the stimulation by $InsP_3$, which is precisely the parameter whose variation induces Ca^{2+} oscillations under physiological conditions.

Bursting and chaos in Ca^{2+} signaling were also shown to occur following alternative scenarios belonging more directly to the first type of mechanism described earlier, i.e., as a result of the interplay between two endogenous oscillatory mechanisms involving two distinct regulatory feedback loops. Thus, in the model of Shen and Larter[22] one oscillatory mechanism involves CICR while the second relies on the possible activation of the synthesis (rather than degradation, as considered above) of $InsP_3$ by Ca^{2+}.[23] In the model of Kummer *et al.*[24] the occurrence of chaotic Ca^{2+} oscillations is based on an interplay between two feedback pro-

cesses involving a receptor-associated G protein and phospholipase C. An alternative model for complex Ca^{2+} oscillations involves Ca^{2+} movements between three pools, namely the endoplasmic reticulum, mitochondria, and cytosolic Ca^{2+}-binding proteins.[54]

The present overview shows that the existence of a multiplicity of regulatory feedback loops in metabolic and genetic control networks creates conditions favorable to the occurrence not only of periodic behavior but also of more complex oscillatory phenomena such as bursting, birhythmicity, and chaos. Besides birhythmicity, other types of coexisting attractors can be observed, such as bistability (coexistence between two stable steady states), and hard excitation (coexistence between a stable steady state and a stable limit cycle). Bistability arising from the control of gene expression (see Ref. 55 for a recent experimental example) likely plays a key role in cell differentiation.[56–58] Bistable behavior could also play a role in all-or-none transitions in regulated metabolic pathways.[59] The molecular mechanisms underlying bistability are closely related to those that produce oscillatory behavior, as shown in a biochemical context by the regulated isocitrate dehydrogenase reaction which displays bistability[60] and which could also provide, in slightly different experimental conditions, a new example of oscillatory enzyme reaction.[61]

From our comparative study of a variety of models based on metabolic or genetic regulation, two main classes of mechanism leading from simple to complex oscillatory behavior have been identified, namely (i) the interplay between two endogenous oscillatory mechanisms, which can take multiple forms, overt or more subtle, depending on whether the two oscillators each involve their own regulatory feedback loop or share a common feedback loop while differing by a process linked to this feedback, and (ii) self-modulation of the oscillator through feedback from the system's output on one of the inputs controlling the very occurrence and characteristics of oscillatory behavior. However, the comparison of the relative sizes of the various domains of dynamic behavior in parameter space shows that even in the presence of either one of these two mechanisms favoring the transition to complex oscillatory behavior, the most common mode of dynamic behavior remains that of simple periodic oscillations.

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