BIOLOGICAL RHYTHMS AS TEMPORAL DISSIPATIVE STRUCTURES

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Special Volume in Memory of Ilya Prigogine: Advances in Chemical Physics, Volume 135, edited by Stuart A. Rice
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I. INTRODUCTION

From the very beginning of his scientific path, which spanned more than six decades, Ilya Prigogine was attracted by the question of how order in time and space spontaneously arises in chemical and biological systems. The title of an article he published in 1969, "Structure, Dissipation and Life," reflects this theme, which long remained a central preoccupation in his research. In this chapter I will show how the views of Ilya Prigogine on nonequilibrium self-organization found multifarious applications in the life sciences. I will focus on temporal self-organization in the form of oscillatory behavior, which is ubiquitous in biological systems. One question that naturally arises is, Why are there so many biological rhythms?

Until the 1950s, the rare periodic phenomena known in chemistry, such as the reaction of Bray [1], represented laboratory curiosities. Some oscillatory reactions were also known in electrochemistry. The link was made between the cardiac rhythm and electrical oscillators [2]. New examples of oscillatory chemical reactions were later discovered [3, 4]. From a theoretical point of view, the first kinetic model for oscillatory reactions was analyzed by Lotka [5], while similar equations were proposed soon after by Volterra [6] to account for oscillations in predator–prey systems in ecology. The next important advance on biological oscillations came from the experimental and theoretical studies of Hodgkin and Huxley [7], which clarified the physicochemical bases of the action potential in electrically excitable cells. The theory that they developed was later applied [8] to account for sustained oscillations of the membrane potential in these cells. Remarkably, the classic study by Hodgkin and Huxley appeared in the same year as Turing's pioneering analysis of spatial patterns in chemical systems [9].

The approach of periodic phenomena in physicochemical terms made further progress when Prigogine and Balescu [10] showed that sustained oscillations can occur far from thermodynamic equilibrium in open chemical systems governed by appropriate, nonlinear kinetic laws. The model analyzed in that study was a chemical analogue of the Lotka–Volterra system for predator–prey oscillations in ecology. The results were later extended by the analysis of abstract models of oscillatory reactions, such as the *Brusselator*, whose name was given by Tyson [11] to a theoretical model studied in detail in Brussels by Lefever, Nicolis, and Prigogine [12]. As shown by these studies, chemical oscillations can occur at a critical distance from equilibrium, around a steady state that has become unstable owing to the presence of autocatalytic steps in the reaction kinetics [12–16].

In the phase space formed by the concentrations of the chemical variables involved in the reaction, sustained oscillations correspond to the evolution towards a closed curve called a limit cycle [17]. The time taken to travel once along the closed curve represents the period of the oscillations. When a single

limit cycle exists, the system always evolves towards the same closed curve characterized by a fixed amplitude and period, for a given set of parameter values, regardless of the initial conditions. It is in this sense that oscillations of the limit cycle type differ from Lotka–Volterra oscillations, for which an infinity of closed curves, corresponding to oscillations of different periods and amplitudes, surround the steady state in the phase space. Then the choice of any one of the closed trajectories depends on the initial conditions [15, 17].

The developments of the Thermodynamics of Irreversible Processes in the nonlinear domain permitted Prigogine to place periodic phenomena within the field of nonequilibrium processes of self-organization [13, 14, 18]. Much as spatial structures arise in chemical systems beyond a critical point of instability with respect to diffusion [9], rhythms correspond to a temporal organization that appears beyond a critical point of instability of a nonequilibrium steady state. These two types of nonequilibrium self-organization represent dissipative structures [13, 14] that can be maintained only by the energy dissipation associated with the exchange of matter between the chemical system and its environment. Sustained oscillations of the limit cycle type can thus be viewed as temporal dissipative structures [13, 14, 15, 18]. When it occurs in constant environmental conditions, periodic behavior provides the clearest sign that a chemical or biological system operates beyond a point of nonequilibrium instability. Endogenous rhythms, produced by a system and not by its environment, are indeed the signature of an instability.

From a mathematical point of view, the onset of sustained oscillations generally corresponds to the passage through a Hopf bifurcation point [19]: For a critical value of a control parameter, the steady state becomes unstable as a focus. Before the bifurcation point, the system displays damped oscillations and eventually reaches the steady state, which is a stable focus. Beyond the bifurcation point, a stable solution arises in the form of a small-amplitude limit cycle surrounding the unstable steady state [15, 17]. By reason of their stability or regularity, most biological rhythms correspond to oscillations of the limit cycle type rather than to Lotka–Volterra oscillations. Such is the case for the periodic phenomena in biochemical and cellular systems discussed in this chapter. The phase plane analysis of two-variable models indicates that the oscillatory dynamics of neurons also corresponds to the evolution toward a limit cycle [20]. A similar evolution is predicted [21] by models for predator–prey interactions in ecology.

The 1970s saw an explosion of theoretical and experimental studies devoted to oscillating reactions. This domain continues to expand as more and more complex phenomena are observed in the experiments or predicted theoretically. The initial impetus for the study of oscillations owes much to the concomitance of several factors. The discovery of temporal and spatiotemporal organization in the Belousov–Zhabotinsky reaction [22], which has remained the most important example of a chemical reaction giving rise to oscillations and waves,

and the elucidation of its reaction mechanism [23] occurred at a time when thermodynamic advances were establishing the theoretical bases of temporal and spatial self-organization in chemical systems under nonequilibrium conditions [10, 13–15, 18].

At the same time as the Belousov–Zhabotinsky reaction provided a chemical prototype for oscillatory behavior, the first experimental studies on the reaction catalyzed by peroxidase [24] and on the glycolytic system in yeast (to be discussed in Section III) demonstrated the occurrence of biochemical oscillations *in vitro*. These advances opened the way to the study of the molecular bases of oscillations in biological systems.

Oscillations represent one of the most striking manifestations of dynamic behavior in biological systems. In 1936, Fessard [25] published a book entitled *Rhythmic Properties of Living Matter*. This book was solely devoted to the oscillatory properties of nerve cells. It has now become clear that rhythms are encountered at all levels of biological organization, with periods ranging from a fraction of a second to years, spanning more than 10 orders of magnitude. The main types of biological rhythms are listed in Table I, where they are ordered according to their period, from the fastest rhythms in nerve and muscle cells to the rhythms of longest period observed in ecology and for the flowering of some plant species.

New examples of cellular rhythms have recently been uncovered (Table II). These include periodic changes in the intracellular concentration of the transcription factor NF-KB and of the tumor suppressors p53, stress-induced oscillations in the transport of the transcription factor Msn2 between cytoplasm and nucleus in yeast, the segmentation clock that is responsible for the

TABLE I
Main Biological Rhythms

Biological Rhythm	Period
Neural rhythms[a]	0.001 s to 10 s
Cardiac rhythm[a]	1 s
Calcium oscillations[a]	sec to min
Biochemical oscillations[a]	30 s to 20 min
Mitotic oscillator[a]	10 min to 24 h
Hormonal rhythms[a]	10 min to 3–5 h (24 h)
Circadian rhythms[a]	24 h
Ovarian cycle	28 days (human)
Annual rhythms	1 year
Rhythms in ecology and epidemiology	years

[a]These rhythms can already occur at the cellular level.

Source: Goldbeter [31].

TABLE II
Some Recently Discovered Cellular Rhythms[a]

Cellular Rhythm	Period
Segmentation clock	90 min
NFκB	3 h
P53	3 h
Msn2 in yeast	6 min
Yeast transcriptome	40 min

[a]See section VIII for details.

formation of somites in vertebrates, and whole genome oscillations in yeast. Some synthetic oscillatory gene circuits were recently constructed, as exemplified by the *Repressilator* [26]. Given the rapidly rising interest in the dynamic behavior of genetic circuits, it is likely that additional examples of cellular rhythms will be found in a near future.

II. DISSIPATIVE STRUCTURES IN TIME AND SPACE

In the course of time open systems that exchange matter and energy with their environment generally reach a stable steady state. However, as shown by Glansdorff and Prigogine, once the system operates sufficiently far from equilibrium and when its kinetics acquire a nonlinear nature, the steady state may become unstable [15, 18]. Feedback regulatory processes and cooperativity are two major sources of nonlinearity that favor the occurrence of instabilities in biological systems.

Some of the main types of cellular regulation associated with rhythmic behavior are listed in Table III. Regulation of ion channels gives rise to the periodic variation of the membrane potential in nerve and cardiac cells [27, 28; for a recent review of neural rhythms see, for example, Ref. 29]. Regulation of enzyme activity is associated with metabolic oscillations, such as those that occur in glycolysis in yeast and muscle cells. Calcium oscillations originate

TABLE III
Biological Regulations and Examples of Associated Cellular Rhythms

Regulation of	Examples of Associated Rhythms
Ion channel	Neural and cardiac rhythms
Enzyme	Glycolytic oscillations in yeast
Receptor	cAMP oscillations in *Dictyostelium*
Transport	Ca^{2+} oscillations
Gene expression	Circadian rhythms, segmentation clock

from the control of transport processes within the cell. Regulation of receptors, coupled to the regulation of enzyme activity, can give rise to periodic behavior, as exemplified by oscillations of cyclic AMP (cAMP) in *Dictyostelium* cells. Regulation of gene expression represents a key type of cellular regulation involved in the mechanism of circadian rhythms and of the segmentation clock.

When the steady state becomes unstable, the system moves away from it and often undergoes sustained oscillations around the unstable steady state. In the phase space defined by the system's variables, sustained oscillations generally correspond to the evolution toward a limit cycle (Fig. 1). Evolution toward a limit cycle is not the only possible behavior when a steady state becomes unstable in a spatially homogeneous system. The system may evolve toward another stable steady state—when such a state exists. The most common case of multiple steady states, referred to as bistability, is of two stable steady states separated by an unstable one. This phenomenon is thought to play a role in differentiation [30]. When spatial inhomogeneities develop, instabilities may lead to the emergence of spatial or spatiotemporal dissipative structures [15]. These can take the form of propagating concentration waves, which are closely related to oscillations.

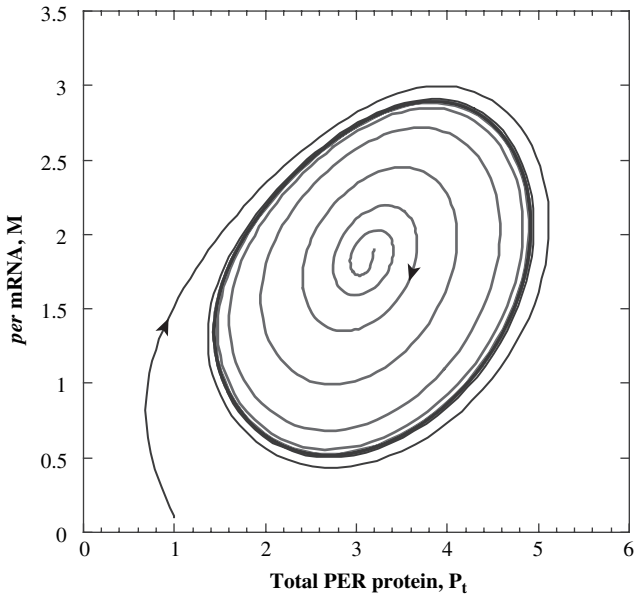

Figure 1. In most examples of biological rhythms, sustained oscillations correspond to the evolution toward a limit cycle. The limit cycle shown here was obtained in a model for circadian oscillations of the PER protein and *per* mRNA in *Drosophila* [107].

Elucidating the molecular mechanism of a biological rhythm largely reduces to identifying the feedback processes that lie at the core of the oscillations. The latter may originate from positive or negative feedback, or from a mixture of both. The interplay between a large number of variables coupled through multiple regulatory interactions makes it difficult, if not impossible, to fully grasp the dynamics of oscillatory behavior without resorting to modeling and computer simulations [31, 32].

As indicated above, theoretical models for biological rhythms were first used in ecology to study the oscillations resulting from interactions between populations of predators and preys [6]. Neural rhythms represent another field where such models were used at an early stage: The formalism developed by Hodgkin and Huxley [7] still forms the core of most models for oscillations of the membrane potential in nerve and cardiac cells [33–35]. Models were subsequently proposed for oscillations that arise at the cellular level from regulation of enzyme, receptor, or gene activity (see Ref. 31 for a detailed list of references).

Some of the main examples of biological rhythms of nonelectrical nature are discussed below, among which are glycolytic oscillations (Section III), oscillations and waves of cytosolic Ca^{2+} (Section IV), cAMP oscillations that underlie pulsatile intercellular communication in *Dictyostelium* amoebae (Section V), circadian rhythms (Section VI), and the cell cycle clock (Section VII). Section VIII is devoted to some recently discovered cellular rhythms. The transition from simple periodic behavior to complex oscillations including bursting and chaos is briefly dealt with in Section IX. Concluding remarks are presented in Section X.

III. GLYCOLYTIC OSCILLATIONS

Glycolytic oscillations in yeast cells provided one of the first examples of oscillatory behavior in a biochemical system. They continue to serve as a prototype for cellular rhythms. This oscillatory phenomenon, discovered some 40 years ago [36, 37] and still vigorously investigated today [38], was important in several respects: First, it illustrated the occurrence of periodic behavior in a key metabolic pathway. Second, because they were soon observed in cell extracts, glycolytic oscillations provided an instance of a biochemical clock amenable to *in vitro* studies. Initially observed in yeast cells and extracts, glycolytic oscillations were later observed in muscle cells and evidence exists for their occurrence in pancreatic β-cells in which they could underlie the pulsatile secretion of insulin [39].

The molecular mechanism of glycolytic oscillations has been discussed for long [31, 38, 40–42]. Because glycolysis represents a system of enzymatic reactions coupled through different intermediates such as ATP and NADH,

which impinge on multiple steps in the pathway, it is difficult to isolate a single enzymatic step that would be responsible for oscillatory behavior. However, there is a large, if not unanimous, consensus in attributing to the enzyme phosphofructokinase (PFK) a prominent role in the instability-generating mechanism that leads to glycolytic oscillations. This role, recognized since the early experimental studies on the phenomenon, is due to the peculiar regulation of PFK, which is activated by a reaction product, ADP. Such product activation means that the PFK reaction is autocatalytic, a feature long shown to be associated with nonequilibrium instabilities [15, 18]. Self-amplification of PFK due to product activation of the enzyme was at the core of early models proposed for glycolytic oscillations [43–45].

A two-variable model taking into account the allosteric (i.e. cooperative) nature of the enzyme and the autocatalytic regulation 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 corresponding to periodic behavior. The model accounts for most experimental data, particularly 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 [31, 45, 46].

Whereas two bifurcation values for the glucose input rate define the domain of oscillations in yeast extracts [40], only a single bifurcation value below which oscillations occur is found in intact yeast cells [47]. This does not necessarily imply a difference in oscillatory mechanism but merely indicates that in intact cells the glucose transporter becomes saturated before the intracellular glucose input has reached the upper bifurcation value above which oscillations disappear in yeast extracts [38].

If the primary role of PFK in generating glycolytic oscillations has long been stressed and substantiated by models based on its regulatory properties, other reactions of the glycolytic pathways are coupled to PFK and may thus influence its dynamic behavior. More complex models incorporating a large number of enzymatic reactions and of glycolytic intermediates have been proposed. This alternative approach to modeling was pioneered more than four decades ago by Garfinkel and Hess [48], who early on presented a comprehensive computer model for the glycolytic pathway. This work represents one of the first studies in a field currently known as (computational) systems biology. Other full-scale models of the yeast glycolytic system were subsequently proposed [49, 50].

The question of how glycolytic oscillations synchronize in a population of yeast cells is of great current interest [51]. It has long been known that the oscillations disappear in a yeast suspension when the cell density decreases below a critical value. Acetaldehyde appears to act as synchronizing factor in such suspensions [52], and the way it allows cells to synchronize is being

studied in both an experimental and theoretical manner. The link between glycolytic oscillations and the pulsatile secretion of insulin in pancreatic β cells [53] is another topic of current concern. Models for the latter phenomenon rely on the coupling between intracellular metabolic oscillations and an ionic mechanism generating action potentials. Such coupling results in bursting oscillations of the membrane potential, which are known to accompany insulin secretion in these cells [54, 55].

IV. CALCIUM OSCILLATIONS

The three best-known examples of biochemical oscillations were found during the decade 1965–1975 [40, 41]. These include the peroxidase reaction, glycolytic oscillations in yeast and muscle, and the pulsatile release of cAMP signals in *Dictyostelium* amoebae (see Section V). Another decade passed before the development of Ca^{2+} fluorescent probes led to the discovery of oscillations in intracellular Ca^{2+}. Oscillations in cytosolic Ca^{2+} have since been found in a variety of cells where they can arise spontaneously, or after stimulation by hormones or neurotransmitters. Their period can range from seconds to minutes, depending on the cell type [56]. The oscillations are often accompanied by propagation of intracellular or intercellular Ca^{2+} waves. The importance of Ca^{2+} oscillations and waves stems from the major role played by this ion in the control of many key cellular processes—for example, gene expression or neurotransmitter secretion.

In cells that use Ca^{2+} as second messenger, binding of an external signal to a cell membrane receptor activates phospholipase C (PLC), which, in turn, synthesizes inositol 1,4,5-trisphosphate ($InsP_3$). This metabolite binds to an $InsP_3$ receptor located on the membrane of internal Ca^{2+} stores (endoplasmic or sarcoplasmic reticulum) and thereby triggers the release of Ca^{2+} into the cytoplasm of the cell [56]. A conspicuous feature of Ca^{2+} release is that it is *self-amplified*: Cytosolic Ca^{2+} triggers the release of Ca^{2+} from intracellular stores into the cytosol, a process known as Ca^{2+}-induced Ca^{2+} release (CICR) [57, 58].

A first model for cytosolic Ca^{2+} oscillations was based on the activation of PLC by Ca^{2+} [59]. Although this positive feedback has been observed in some cell types, CICR represents a more general self-amplifying process underlying the oscillations. Several processes limit the explosive nature of self-amplification. A simple two-variable model for signal-induced Ca^{2+} oscillations based on CICR accounts for oscillations of cytosolic Ca^{2+} [60]. Sustained oscillations occur between two critical values of the stimulus intensity—for example, two critical levels of an extracellular hormonal signal (see Fig. 2). Below the lower critical value, a low steady-state level of cytosolic Ca^{2+} is established; above the larger critical value, the system evolves toward a higher, stable steady-state level

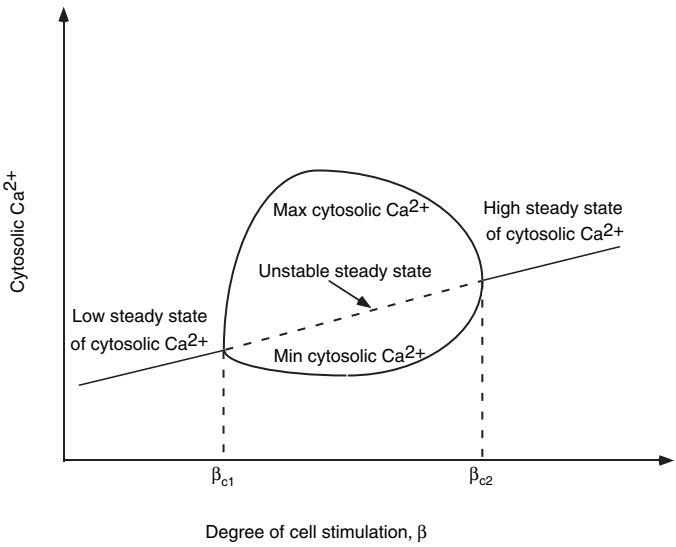

Figure 2. Schematic bifurcation diagram showing the domain and amplitude of intracellular Ca^{2+} oscillations as a function of the degree of external stimulation, β, which is used as control parameter. Sustained Ca^{2+} oscillations occur in a range of stimulation between the two critical β values denoted β_{c1} and β_{c2}. The maximum and minimum of cytosolic Ca^{2+} oscillations are plotted as a function of β in this range, in which the dashed line refers to the unstable steady state. On the left and right sides of the oscillatory domain, the system evolves to a stable steady state (solid line) corresponding to a low and high level of cytosolic Ca^{2+}, respectively. The bifurcation diagram is obtained in a two-variable model for Ca^{2+} oscillations based on CICR (see Goldbeter et al. [60] for a nonschematic version of the diagram). A similar bifurcation diagram with a domain of sustained oscillations bounded by two bifurcation values of the control parameter is obtained for glycolytic oscillations as a function of the substrate injection rate in yeast extracts [46, 193]. In intact yeast cells, however, the upper bifurcation point cannot be reached, likely because, as described in Section III, the glucose transporter is saturated before the bifurcation value for the substrate input is reached inside the cell.

of cytosolic Ca^{2+}. The model predicts that the frequency of Ca^{2+} oscillations rises with the degree of stimulation, as observed experimentally. In this minimal model the level of intracellular $InsP_3$ is treated as a control parameter reflecting the degree of external stimulation. More complex models for Ca^{2+} oscillations are based on more detailed descriptions of $InsP_3$ receptor kinetics [61; for a recent review see Ref. 62] but still attribute to CICR a primary role in the origin of repetitive Ca^{2+} spiking.

Mathematical models for Ca^{2+} signaling were subsequently developed in two additional directions. First, waves of intra- or intercellular Ca^{2+} can be modeled by incorporating the diffusion of cytosolic Ca^{2+} or the passage of Ca^{2+} or $InsP_3$ from cell to cell through gap junctions [62–65]. While most models for

Ca^{2+} waves are deterministic, stochastic simulations were used to clarify the nature of local increases of cytosolic Ca^{2+} known as blips or puffs which are thought to trigger the onset of waves [56, 66]. Second, models are used to probe mechanisms for encoding Ca^{2+} spikes in terms of their frequency. A variety of physiological responses are controlled by the frequency and waveform of Ca^{2+} oscillations, such as gene expression during development [67]. Among the processes that could underlie such frequency encoding are protein (de)phosphorylation by a Ca^{2+}-dependent kinase (phosphatase) [60], or the Ca^{2+}-dependence of calmodulin-kinase II [68, 69]. A study combining experimental and modeling approaches showed the possibility of frequency encoding of Ca^{2+} spikes by interplay with cyclic AMP signaling [70].

The characteristics of cytosolic Ca^{2+} oscillations vary from one cell type to another. One source for this variability is the existence of three isoforms of the inositol 1,4,5-trisphosphate receptor, $InsP_3R$, whose proportions vary in different cells. Upon binding of $InsP_3$, the $InsP_3R$ functions as a Ca^{2+} channel on the endoplasmic reticulum, allowing passage of Ca^{2+} into the cytoplasm of the cell. The relative amounts of each isoform of the $InsP_3$ receptor affect the time course of Ca^{2+} changes after agonist stimulation, because the effect of Ca^{2+} on the three isoforms are different. The different modes of Ca^{2+} oscillatory behavior have recently been modeled as a function of the relative proportions of the three $InsP_3R$ isoforms [71]. Based on a comparative study of various models, Sneyd et al. [72] recently proposed a method for determining the dependence of Ca^{2+} oscillations on $InsP_3$ oscillations, to determine whether $InsP_3$ plays an active role in the oscillatory mechanism or passively follows the periodic spikes in Ca^{2+}.

The role of Ca^{2+} oscillations in some physiological disorders begins to be characterized. Two recent studies provide examples of how changes in Ca^{2+} oscillatory signaling possess profound implications for developmental processes. First, Beltramello et al. [73] showed that a mutation associated with hereditary deafness reduces metabolic coupling mediated by $InsP_3$ and impairs the propagation of intercellular Ca^{2+} waves. Second, Uhlen et al. [74] recently demonstrated that the Noonan syndrome, a human developmental disorder often accompanied by congenital heart abnormalities, is caused by alterations in the Ca^{2+} oscillatory control of the transcription factor NFAT. These examples illustrate the impact of changes in the normal patterns of oscillations and waves of Ca^{2+} on the pathogenesis of some inherited human diseases.

V. PULSATILE INTERCELLULAR COMMUNICATION IN *DICTYOSTELIUM*

While intracellular information can be encoded in the frequency of signal-induced Ca^{2+} spikes, some extracellular signals can themselves be produced in a

periodic, pulsatile manner. Examples of pulsatile intercellular communication include episodic hormone secretion and pulsatile signals of cAMP in the slime mold *Dictyostelium discoideum*. The latter phenomenon represents a prototype both for spatiotemporal self-organization and for pulsatile signaling in intercellular communication [31].

A. Oscillations of cAMP

After starvation, *Dictyostelium* amoebae undergo a transition from a unicellular to a multicellular phase of their life cycle. By a chemotactic response to cAMP signals, up to 10^5 amoebae collect around cells behaving as aggregation centers. These centers release cAMP with a period of about 5 min; surrounding cells relay the chemotactic signal toward the periphery of the aggregation field. Relay and oscillations of cAMP result in the formation of concentric or spiral waves of aggregating cells [75].

Models help to clarify the mechanism of cAMP oscillations in *Dictyostelium* [76, 77]. The mechanism involves both positive and negative feedback. Binding of extracellular cAMP to a cell surface receptor leads to the activation of adenylate cyclase, which catalyzes the synthesis of intracellular cAMP. Transport of cAMP into the extracellular medium creates a positive feedback loop, which elicits a rapid rise in cAMP synthesis. For sustained oscillations to occur, this rise in cAMP must be self-limiting, so that cAMP first levels off before decreasing to its minimum level. Models confirm that negative feedback due to cAMP-induced receptor desensitization through reversible phosphorylation can play such a role in limiting self-amplification [76]. Once the levels of intra- and extracellular cAMP are sufficiently low, dephosphorylation can resensitize the receptor. The ensuing buildup of extracellular cAMP progressively brings it to the threshold above which self-amplification triggers a new pulse.

Numerical simulations indicate that relay of cAMP pulses represents a different mode of dynamic behavior, closely related to oscillations. Just before autonomous oscillations break out, cells in a stable steady state can amplify suprathreshold variations in extracellular cAMP in a pulsatory manner. Thus, relay and oscillations of cAMP are produced by a unique mechanism in adjacent domains in parameter space. The two types of dynamic behavior are analogous to the excitable or pacemaker behavior of nerve cells.

Theoretical models shed light on additional aspects of pulsatile cAMP signaling in *Dictyostelium*. First, like Ca^{2+} spikes, cAMP pulses are frequency encoded. Only pulses delivered at 5-min intervals are capable of accelerating slime mold development after starvation. Simulations indicate that frequency encoding is based on reversible receptor desensitization [76]. The kinetics of receptor resensitization dictates the interval between successive pulses required for a maximum relay response [78]. Second, cAMP oscillations in

Dictyostelium provide a prototype for the ontogenesis of biological rhythms. The amoebae become capable of relaying extracellular cAMP pulses only a few hours after the beginning of starvation, before acquiring the property of autonomous oscillations. Models show that these developmental transitions can be brought about by the continuous increase in certain biochemical parameters such as the activities of adenylate cyclase or phosphodiesterase, the enzyme that degrades cAMP. In parameter space, these biochemical changes define a *developmental path* that successively crosses domains corresponding to different types of dynamic behavior, from no relay to relay, and finally to oscillations [31, 79].

Models are also being used to probe the mechanisms underlying the formation of concentric or spiral waves of cAMP responsible for the spatiotemporal patterns observed during aggregation [80]. Among the factors shown to play a role in the transition between the two types of waves are the activity of extracellular phosphodiesterase [81] and desynchronization of the cells that follow the developmental path after starvation [82]. The model based on the positive feedback mechanism coupled to receptor desensitization also accounts for the propagation of planar and scroll waves within the multicellular slug formed by the amoebae after aggregation [83].

In recent years, work by Loomis and co-workers has raised the possibility that cAMP oscillations in *D. discoideum* may originate from an intracellular regulatory network rather than from the mixed positive and negative feedback exerted by extracellular cAMP [84, 85]. These authors obtained evidence for an intracellular feedback loop involving MAP kinase and the cAMP-dependent protein kinase, PKA. The later enzyme would inactivate adenylate cyclase after a cAMP pulse. Numerical simulations of a model based on this intracellular negative feedback loop confirm that it can produce sustained oscillations of cAMP.

To establish which of the two feedback loops plays a prominent role in the origin of cAMP oscillations, Cox and co-workers recently examined the patterns of wavelike aggregation in a variety of mutants lacking components of the intracellular and extracellular regulatory loops. They reached the conclusion that the primary (but not necessarily sole) source of the oscillations resides in the regulation exerted by extracellular cAMP upon binding to its membrane receptor [86]. Interestingly, the possibility of cAMP oscillations due to intracellular regulation of adenylate cyclase by PKA seems to exist not only in *Dictyostelium* but also in yeast. In this organism, Jacquet et al. [87] recently observed a stress-induced oscillatory shuttling of the transcription factor Msn2 between cytosol and nucleus. They since obtained evidence suggesting that this periodic phenomenon is caused by intracellular cAMP oscillations, via the control of adenylate cyclase by PKA (see Section VIII). In this view, periodic activation of PKA by cAMP oscillations in yeast would

underlie the repetitive, coherent shuttling of the transcription factor Msn2 into and out of the nucleus.

B. Link with Pulsatile Hormone Secretion

Pulsatile cAMP signaling in *Dictyostelium* is closely related with pulsatile hormone secretion in higher organisms. It is now clear that most hormones are secreted in a pulsatile rather than continuous manner [88] and that the temporal pattern of a hormone is often as important as its concentration in the blood [89]. The best examples of pulsatile hormone secretion are the gonadotropin-releasing hormone (GnRH) released by the hypothalamus with a periodicity of 1 h in man and rhesus monkey [90], the growth hormone (GH) secreted by the hypothalamus with a period of 3–5 h [91], and insulin secreted by pancreatic β cells with a period close to 13 min in man [53]. In the cases of GnRH and GH—the effect is less clear-cut for insulin—the frequency of the pulses governs the physiological efficacy of hormone stimulation [90, 91].

A general model for a two-state receptor subjected to periodic ligand variations shows that frequency encoding of hormone pulses may rely on reversible desensitization in target cells, as in the case of cAMP pulses in *Dictyostelium* [78, 92]. The mechanism of the hypothalamic GnRH pulse generator is still unknown and provides an important challenge for both experiments and theory. The basis of pulsatile GH secretion has been studied by a modeling approach [93]. In β cells, pulsatile insulin release could originate from insulin feedback on glucose transport into the cells [94] or from oscillatory membrane activity driven by glycolytic oscillations [53–55]. Together with such metabolic oscillations, membrane potential bursting and Ca^{2+} oscillations in β cells illustrate the multiplicity of rhythms that can be encountered in a given cell type.

VI. CIRCADIAN RHYTHMS

The most ubiquitous biological rhythms are those that occur with a period close to 24 h in all eukaryotes and in some prokaryotes such as cyanobacteria. These circadian rhythms allow organisms to adapt to the natural periodicity of the terrestrial environment, which is characterized by the alternation of day and night due to rotation of the earth on its axis. Circadian clocks provide cells with an endogenous mechanism, allowing them to anticipate the time of day.

Experimental advances during the last decade have clarified the molecular bases of circadian rhythms, first in *Drosophila* and *Neurospora*, and more recently in cyanobacteria, plants, and mammals [95–99]. In nearly all cases investigated so far, it appears that circadian rhythms originate from the negative

feedback exerted by a protein on the expression of its gene [100]. Circadian rhythms in cyanobacteria appear to be based on a different molecular mechanism, which can be uncoupled from transcriptional control. Thus the circadian oscillation in the phosphorylation of the cyanobacterial KaiC clock protein has recently been reconstituted *in vitro* [101].

Before details on the molecular mechanism of circadian rhythms began to be uncovered, theoretical models borrowed from physics were used to investigate the dynamic properties of circadian clocks. The relative simplicity of these models explains why their use continues to this day. Thus, the Van der Pol equations, derived for an electrical oscillator, served for modeling the response of human circadian oscillations to light [102] and to account for experimental observations on increased fitness due to resonance of the circadian clock with the external light–dark (LD) cycle in cyanobacteria [103, 104]. The earliest model predicting oscillations due to negative feedback was proposed by Goodwin [105], at a time when the role played by such a regulatory mechanism in the origin of circadian rhythms was not yet known. Models based on Goodwin's equations are still being used in studies of circadian oscillations—for example, in *Neurospora* [106].

A. Circadian Rhythms in *Drosophila*

Molecular models for circadian rhythms were initially proposed [107] for circadian oscillations of the PER protein and its mRNA in *Drosophila*, the first organism for which detailed information on the oscillatory mechanism became available [100]. The case of circadian rhythms in *Drosophila* illustrates how the need to incorporate experimental advances leads to a progressive increase in the complexity of theoretical models. A first model governed by a set of five kinetic equations is shown in Fig. 3A; it is based on the negative control exerted by the PER protein on the expression of the *per* gene [107]. Numerical simulations show that for appropriate parameter values, the steady state becomes unstable and limit cycle oscillations appear (Fig. 1).

The early model based on PER alone did not account for the effect of light on the circadian system. Experiments subsequently showed that a second protein, TIM, forms a complex with PER and that light acts by inducing TIM degradation [96]. An extended, 10-variable model was then proposed [108], in which the negative regulation is exerted by the PER–TIM complex (Fig. 3B). This model produces essentially the same result, sustained oscillations in continuous darkness. In addition, it accounts for the behavior of mutants and explicitly incorporates the effect of light on the TIM degradation rate. Thereby the model can account for the entrainment of the oscillations by light-dark (LD) cycles and for the phase shifts induced by light pulses.

Subsequent experimental studies have shown that the mechanism of circadian rhythms in *Drosophila* is more complex, since the negative

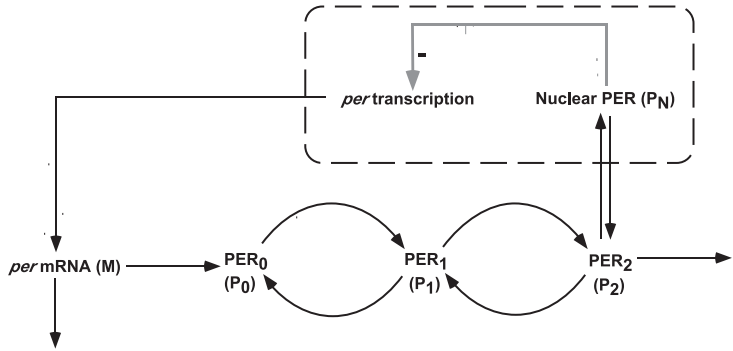

Figure 3. Molecular models of increasing complexity considered for circadian oscillations. (A) Model for circadian oscillations in *Drosophila* based on negative autoregulation of the *per* gene by its protein product PER [31, 107]. The model incorporates gene transcription into *per* mRNA, transport of *per* mRNA into the cytosol as well as mRNA degradation, synthesis of the PER protein at a rate proportional to the *per* mRNA level, reversible phosphorylation and degradation of PER, and transport of PER into the nucleus where it represses the transcription of the *per* gene. The model is described by a set of five kinetic equations. (B) Model for circadian oscillations in *Drosophila* incorporating the formation of a complex between the PER and TIM proteins [108]. The model is described by a set of 10 kinetic equations. (C) Model for circadian oscillations in mammals incorporating indirect, negative autoregulation of the *Per* and *Cry* genes through binding of the PER-CRY dimer to the complex formed between the two activating proteins CLOCK and BMAL1. Also considered is the negative feedback exerted by the latter proteins on the expression of their genes. Synthesis, reversible phosphorylation, and degradation of the various proteins are taken into account. The model is described by a set of 16 kinetic equations, or 19 when the Rev-Erbα gene is incorporated into the model [114]. For appropriate parameter values, all three models admit sustained circadian oscillations in conditions corresponding to continuous darkness. The effect of light is taken into account in models (B) and (C) by incorporating light-induced TIM degradation or light-induced *Per* expression, respectively.

autoregulation exerted by the PER–TIM complex on gene expression is indirect (see below).

B. The Mammalian Circadian Clock

The pacemaker generating circadian rhythms in mammals is located in the suprachiasmatic nuclei of the hypothalamus. Recent studies have shown, however, that a number of peripheral circadian oscillators operate in tissues such as liver and heart [109]. Theoretical models for circadian rhythms in *Drosophila* bear on the mechanism of circadian oscillations in mammals, where homologues of the *per* gene exist and negative autoregulation of gene expression is also found [96]. However, in mammals, the role of TIM as a partner for PER is played by the CRY protein, and light acts by inducing gene expression rather than protein degradation as in *Drosophila*. A further analogy between

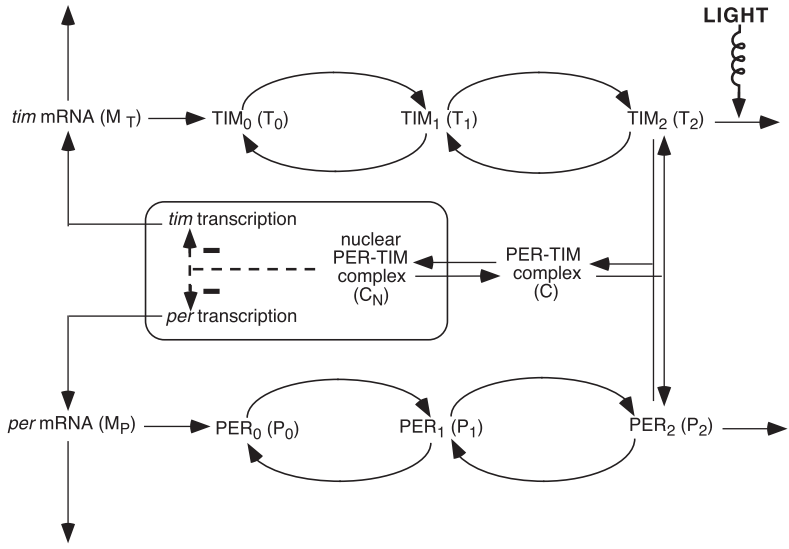

Figure 3. (*Continued*)

Drosophila and mammals is that the negative feedback on gene expression is indirect: The PER-TIM or PER-CRY complexes exert their repressive effect by binding to a complex of two proteins, CLOCK-CYC or CLOCK-BMAL1 in the fly [110] and in mammals [111], respectively. These proteins activate *per* and *tim* (or *cry*) gene expression. Thus negative feedback occurs by counteracting the effect of gene activators. Additional feedback loops are present, such as the negative feedback exerted by CLOCK or BMAL1 on the expression of their genes. These controls are removed upon formation of the complex with the PER-TIM or PER-CRY dimers.

Further extensions of the model are required to address the dynamical consequences of these additional regulatory loops and of the indirect nature of the negative feedback on gene expression. Such extended models have been proposed for *Drosophila* [112, 113] and mammals [113]. The model for the circadian clock mechanism in mammals is schematized in Fig. 3C. The presence of additional mRNA and protein species, as well as of multiple complexes formed between the various clock proteins, complicates the model, which is now governed by a system of 16 or 19 kinetic equations. Sustained or damped oscillations can occur in this model for parameter values corresponding to continuous darkness. As observed in the experiments on the mammalian clock, *Bmal1* mRNA oscillates in opposite phase with respect to *Per* and *Cry* mRNAs [97]. The model displays the property of entrainment by the LD cycle

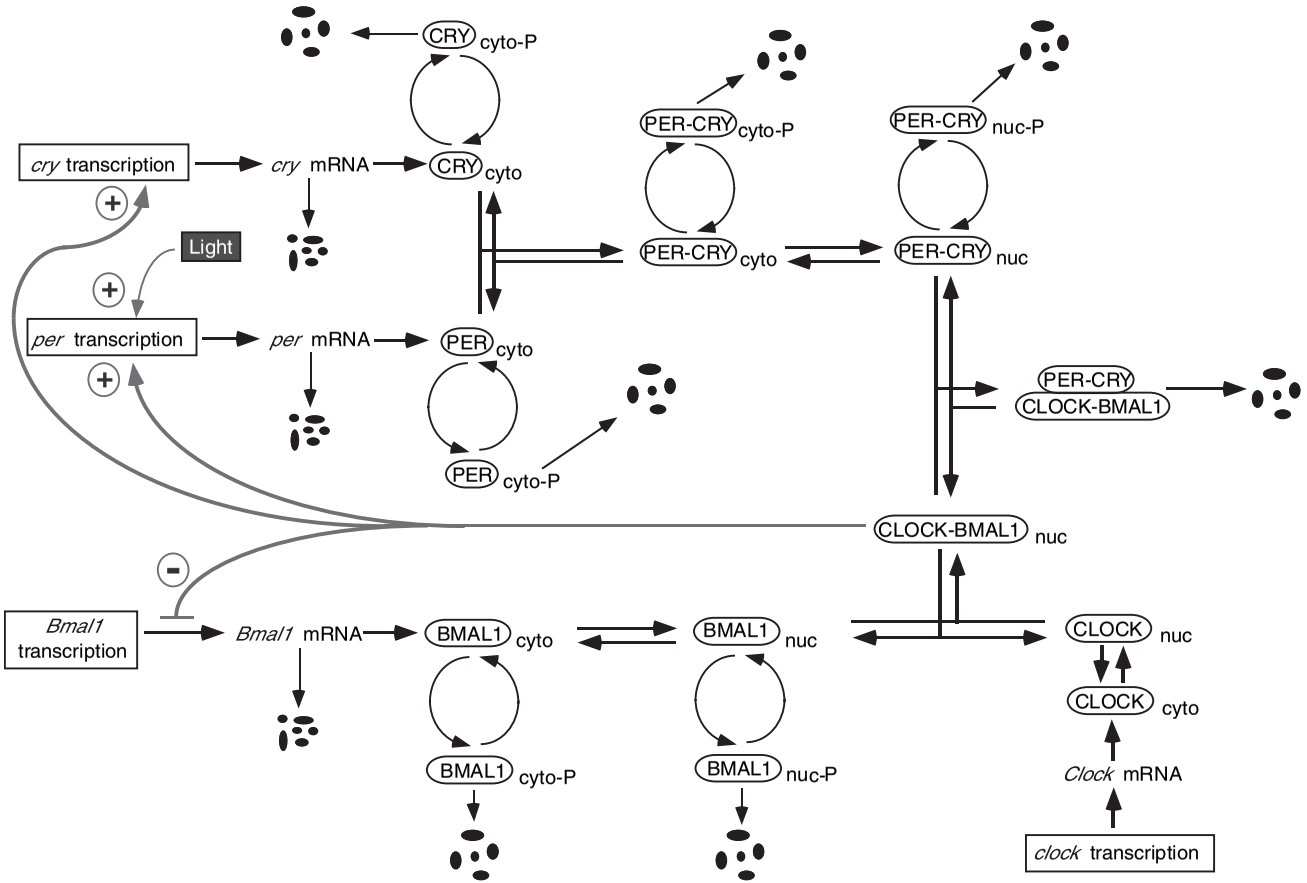

Figure 3. (*Continued*)

when incorporating the light-induced increase in the rate of *Per* expression. A more detailed model containing a much larger number of variables has been proposed for the mammalian circadian clock [115].

Knowledge of the detailed mechanism underlying circadian rhythms continues to be refined as new experiments reveal novel facets of the oscillatory machinery. Thus, a link has recently been established between chromatin structure and the circadian oscillatory mechanism. The CLOCK protein indeed functions as a histone acetyltransferase [116]. This enzyme activity is required for oscillations so that histone modification and the associated chromatin remodeling are implicated in the origin of circadian rhythmicity.

C. Link with Disorders of the Sleep–Wake Cycle

The results obtained with the model for the mammalian circadian clock provide cues for circadian-rhythm-related sleep disorders in humans [117]. Thus permanent phase shifts in LD conditions could account for (a) the familial advanced sleep phase syndrome (FASPS) associated with PER hypophosphorylation [118, 119] and (b) the delayed sleep phase syndrome, which is also related to PER [120]. People affected by FASPS fall asleep around 7:30 P.M. and awake around 4:30 A.M. The duration of sleep is thus normal, but the phase is advanced by several hours. Moreover, the autonomous period measured for circadian rhythms in constant conditions is shorter [121]. The model shows that a decrease in the activity of the kinase responsible for PER phosphorylation is indeed accompanied by a reduction of the circadian period in continuous darkness and by a phase advance upon entrainment of the rhythm by the LD cycle [114].

For some parameter values the model for the mammalian clock fails to allow entrainment by 24-h LD cycles, regardless of the amplitude of the light-induced change in *Per* expression. The question arises whether there exists a syndrome corresponding to this mode of dynamic behavior predicted by the model. Indeed there exists such a syndrome, known as the non-24-h sleep–wake syndrome, in which the phase of the sleep–wake pattern continuously varies with respect to the LD cycle; that is, the patient free-runs in LD conditions [117]. Disorders of the sleep–wake cycle associated with alterations in the dynamics of the circadian clock belong to the broad class of ''dynamical diseases'' [122, 123], although the term ''syndrome'' seems more appropriate for some of these conditions.

Another common perturbation of the circadian clock is the jet lag, which results from an abrupt shift in the phase of the LD cycle to which the rhythm is naturally entrained. The molecular bases of the jet lag are currently being investigated [124]. The model for the circadian clock is being used to probe the various ways by which the clock returns to the limit cycle trajectory after a sudden shift in the phase of the LD cycle.

D. Long-Term Suppression of Circadian Rhythms by a Single Light Pulse

Circadian rhythms illustrate how theoretical models can provide surprising, counterintuitive insights. A case in point is the puzzling observation that in some organisms, circadian rhythms in continuous darkness can be suppressed by a single pulse of light and restored by a second such pulse. A first theoretical explanation for this long-term suppression, proposed by Winfree [125], assumes that the limit cycle in each oscillating cell surrounds an unstable steady state. The light pulse would act as a critical perturbation that would bring the clock to the singularity—that is, the steady state. Because the steady state is unstable, each cell would eventually return to the limit cycle, but with a random phase. The population of oscillating cells would then be spread out over the entire cycle so that the cells would be desynchronized and no global rhythm would be established.

An alternative explanation is based on the *coexistence* of sustained oscillations with a stable steady state. Such coexistence has been observed [126] in the model for circadian rhythms in *Drosophila* based on negative autoregulation by the PER-TIM complex (Fig. 3B). In such a situation, the effect of the light pulse is to bring the clock mechanism into the basin of attraction of the stable steady state in each oscillating cell, so that the rhythm is suppressed. A second light pulse then brings the system back to the basin of attraction of the limit cycle corresponding to circadian oscillations. Without a model it is impossible to predict the coexistence between a stable steady state and a stable rhythm. The question remains open as to which one of the two explanations accounts for long-term suppression of circadian rhythms by a single light pulse.

E. Stochastic Versus Deterministic Models for Circadian Rhythms

Only deterministic models for cellular rhythms have been discussed so far. Do such models remain valid when the numbers of molecules involved are small, as may occur in cellular conditions? Barkai and Leibler [127] stressed that in the presence of small amounts of mRNA or protein molecules, the effect of molecular noise on circadian rhythms may become significant and may compromise the emergence of coherent periodic oscillations. The way to assess the influence of molecular noise on circadian rhythms is to resort to stochastic simulations [127–129]. Stochastic simulations of the models schematized in Fig. 3A,B show that the dynamic behavior predicted by the corresponding deterministic equations remains valid as long as the maximum numbers of mRNA and protein molecules involved in the circadian clock mechanism are of the order of a few tens and hundreds, respectively [128]. In the presence of molecular noise, the trajectory in the phase space transforms into a cloud of points surrounding the deterministic limit cycle.

Stochastic simulations confirm the existence of bifurcation values of the control parameters bounding a domain in which sustained oscillations occur. The effect of noise diminishes as the number of molecules increases. Only when the maximum numbers of molecules of mRNA and protein become smaller than a few tens does noise begin to obliterate the circadian rhythm. The robustness of circadian rhythms with respect to molecular noise is enhanced when the rate of binding of the repressor molecule to the gene promoter increases [128]. Conditions that enhance the resistance of genetic oscillators to random fluctuations have been investigated [130].

VII. THE CELL-CYCLE CLOCK

The cell cycle is a key process that recurs in a periodic manner. Early cell cycles in amphibian embryos are driven by a mitotic oscillator. This oscillator produces the repetitive activation of the cyclin-dependent kinase cdk1, also known as cdc2 [131]. Cyclin synthesis is sufficient to drive repetitive cell division cycles in amphibian embryonic cells [132]. The period of these relatively simple cell cycles is of the order of 30 min. In somatic cells the cell cycle becomes longer, with durations of up to 24 h or more, owing to the presence of checkpoints that ensure that a cell cycle phase is properly completed before the cell progresses to the next phase. The cell cycle goes successively through the phases G1, S (DNA replication), G2, and M (mitosis) before a new cycle starts in G1. After mitosis cells can also enter a quiescent phase G0, from which they enter G1 under mitogenic stimulation.

Models of reduced complexity have first been proposed for the early cell cycles in amphibian embryos. These models are based on the activation of the kinase cdc2 upon binding of cyclin. One of these models predicts that limit cycle oscillations in cdc2 activity may arise from the activation by cdc2 of cyclin degradation. Indeed, cdc2 activates the anaphase-promoting complex (APC), which leads to cyclin destruction and subsequently to cdc2 inactivation. Such a negative feedback regulation is capable of producing sustained oscillatory behavior in the presence of thresholds and delays, both of which are linked and naturally arise in the control of cdc2 by phosphorylation-dephosphorylation [31, 133].

Positive feedback is also involved in the control of cdc2 by reversible phosphorylation. Thus, cdc2 activates the phosphatase cdc25, which catalyzes the dephosphorylation and concomitant activation of the kinase cdc2. The model based on negative feedback in cyclin-cdc2 interactions can be extended to take this positive feedback into account. Sustained oscillations can be obtained in these conditions [31, 134], but the waveform of cdc2 in the course of oscillations now displays a plateau. This plateau is due to the occurrence of a phenomenon of bistability, which is accompanied by hysteresis, as

shown theoretically and experimentally in *Xenopus* egg extracts by Pomerening et al. [135] and Sha et al. [136]. The effect of suppressing the positive feedback loop on the occurrence of cdc2 oscillations was investigated in recent experiments [137].

The interplay between oscillations and bistability has been addressed in detailed molecular models for the cell cycles of amphibian embryos, yeast and somatic cells [138–141]. The predictions of a detailed model for the cell cycle in yeast were successfully compared with observations of more than a hundred mutants [142]. Other theoretical studies focus on the dynamical properties of particular modules of the cell cycle machinery such as that controlling the G1/S transition [143].

If the cell cycle in amphibian embryonic cells appears to be driven by a limit cycle oscillator, the question arises as to the precise dynamical nature of more complex cell cycles in yeast and somatic cells. Novak et al. [144] constructed a detailed bifurcation diagram for the yeast cell cycle, piecing together the diagrams obtained as a function of increasing cell mass for the transitions between the successive phases of the cell cycle. In these studies, cell mass plays the role of control parameter; a critical mass has to be reached for cell division to occur, provided that it coincides with a surge in cdk1 activity which triggers the G2/M transition.

The periodic recurrence of cell division suggests that globally the cell cycle functions like an autonomous oscillator. An extended model incorporating the sequential activation of the various cyclin-dependent kinases, followed by their inactivation, shows that even in the absence of control by cell mass, this sequence of biochemical events can operate as a limit cycle oscillator [145]. This supports the union of the two views of the cell cycle as dominoes and clock [146]. Because of the existence of checkpoints, however, the cell cycle stops at the end of certain phases before engaging in the next one. Thus the cell cycle looks more like an oscillator that slows down and makes occasional stops. A metaphor for such behavior is provided by the movement of the round plate on the table in a Chinese restaurant, which would rotate continuously under the movement imparted by the participants, were it not for frequent stops.

An alternative approach for modeling the cell cycle considers the sequential transitions between the G1, S, G2, and M phases without taking into account the underlying molecular mechanism. Based on a previous study of the dynamics of hair cycles [147], this phenomenological approach represents the cell cycle as a stochastic automaton capable of switching between the successive phases, with a probability related to their duration. The automaton model can reproduce the distributions between the various phases of the cell cycle at steady state [148]. This phenomenological model is being used to investigate the effect of periodic administration of anticancer drugs that interfere with the cell division cycle.

Recent experimental studies have uncovered a direct link between the cell cycle and circadian rhythms. Thus, the circadian clock protein BMAL1 induces the expression of the gene *Wee1*, which codes for the protein kinase that inactivates through phosphorylation the kinase cdk1 that controls the G2/M transition [149]. This link allows the coupling of cell division to the circadian clock and explains how the latter may entrain the cell cycle clock in a variety of cell types.

VIII. NEWLY DISCOVERED CELLULAR RHYTHMS

In the last decade, and particularly in the last five years, several new examples of cellular rhythms have been uncovered (see Table II). These include oscillations in the tumor suppressor p53 and in the transcription factor NF-KB, the segmentation clock that controls the formation of somites in vertebrates, and the oscillatory nucleocytoplasmic shuttling of the transcription factor Msn2 in yeast. Other examples are the genome-wide periodicity of about 80 min observed for gene expression in yeast [150, 151] and the ''transcriptional clock'' based on the estrogen-receptor-mediated ordered, cyclical recruitment of protein cofactors involved in target gene transcription [152].

A. Oscillations of p53 and NF-KB

The tumor suppressor p53 plays an important role in the control of the cell cycle, and it is inactivated in many types of human tumors. In response to genomic stress, p53 activation may elicit cell-cycle arrest or apoptotic cell death, as well as contribute to DNA repair processes. Regulation of p53 is mediated by its interactions with the protein Mdm2. The binding of Mdm2 to p53 inhibits the transcriptional functions of p53 and also leads to its degradation. At the same time, p53 stimulates the transcription of the *mdm2* gene. These interactions define a negative feedback loop ensuring that the p53 response is brought to an end once a p53-activating stress signal has been effectively dealt with.

The dynamics of the p53-Mdm2 feedback loop was analyzed mathematically by Lev Bar-Or et al. [153], who pointed out that this negative feedback regulation can give rise to oscillatory behavior if there is a sufficient delay in the induction of Mdm2 by p53. They verified experimentally that oscillations of both p53 and Mdm2 indeed occur on exposure of various cell types to ionizing radiation. Lahav et al. [154] pursued this study by investigating the dynamics of p53 and Mdm2 in individual cells. They showed that p53 was expressed in a series of discrete pulses after DNA damage. The number of p53 pulses, but not their amplitude, varied in different cells and increased with DNA damage. Ma et al. [155] recently proposed a model for this ''digital'' response of individual cells to DNA damage. The model is based on the coupling of DNA

damage to the p53-Mdm2 oscillator. An alternative model for oscillations in the p53/Mdm2 module has also been proposed [156].

A negative feedback loop likewise controls the activity of the transcription factor NF-KB, which is rapidly turned off by a protein inhibitor, I-KB, which exists under three isoforms denoted α, β, and ε. Only the I-KBα isoform participates in the negative feedback. When NF-KB dissociates from I-KB in the cytosol, it enters the nucleus, where it induces the transcription of a number of target genes, one of which codes for the inhibitor I-KBα. Hoffmann et al. [157] analyzed a computational model based on the interactions of NF-KB and its inhibitor I-KBα. They predicted and verified experimentally that these interactions can give rise to oscillatory changes in NF-KB activity characterized by a period of the order of several hours. Using single-cell time-lapse imaging and computational modeling, Nelson et al. [158] showed that NF-KB localization oscillates between the nucleus and the cytosol following cell stimulation. The frequency of these oscillations decreased with increased I-KBα transcription. The question of whether the pattern of NF-KB oscillations can selectively control the expression of certain genes remains open [157–159].

B. Segmentation Clock

The formation of somites in the course of vertebrate development is associated with body segmentation. This phenomenon represents a striking example of spatial pattern in morphogenesis. It has long been suggested that a temporal periodic process is also at work in somitogenesis, since pairs of somites form progressively, one at a time, along the presomitic mesoderm (PSM). Thus Cooke and Zeeman [160] proposed a ''clock and wavefront'' model, which postulated the existence of (a) a wavefront moving from the anterior to the posterior end of the PSM and (b) a clock that would periodically induce the formation of a pair of somites at the location of the wavefront. Neither the nature of the clock nor the wavefront-defining process was characterized in this abstract model, which nevertheless proved highly seminal. Experimental evidence for an oscillator involved in somitogenesis was later obtained [161, 162]. This oscillator is based on periodic gene expression and is known as the vertebrate ''segmentation clock'' [163–165]. Its period is of the order of 90 min to 2 h, depending on the organism considered. A unique feature of the segmentation clock is that is links a temporal oscillation with the formation of a stable spatial pattern, in an important developmental process [163].

The mechanism of the segmentation clock relies on the negative feedback exerted on the expression of genes that participate in the signaling pathway controlled by Notch and other transcription factors [163-165]. Thus, periodic Notch inhibition by the product of the gene *lunatic fringe* (*Lfng*) underlies the chick segmentation clock [166]. The product of *Lfng* establishes a negative feedback loop that results in the periodic inhibition of Notch, which, in turn,

controls the rhythmic expression of cyclic genes in the chick PSM. This feedback loop provides a molecular basis for the oscillator underlying the avian segmentation clock. A model based on such negative autoregulatory feedback on gene expression (Fig. 4) confirms that it can produce sustained oscillations [167].

Subsequent experimental studies have shown that another signal pathway controlled by WNT may drive oscillations in the Notch pathway [168]. The Wnt pathway is also regulated by negative feedback and could thus give rise to oscillatory behavior (Fig. 5), as shown by a modeling study [167]. Oscillations could be transduced from one pathway to the other by a common intermediate such as the protein kinase GSK3, which is involved both in Wnt and Notch signaling.

There is thus a multiplicity of negative feedback processes that could, in principle, give rise to oscillations in Notch signaling, and on which the

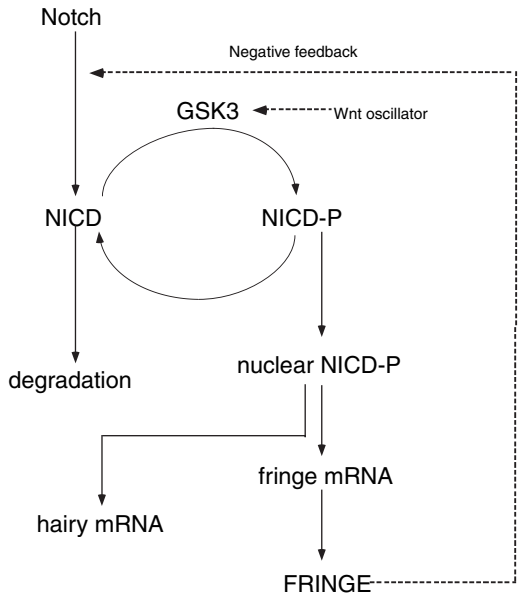

Figure 4. A negative feedback mechanism in the Notch signaling pathway can give rise to periodic expression of genes such as *Lunatic fringe* (*Lnfg*). This negative feedback on transcription is thought to play a key role in the segmentation clock controlling somatogenesis in vertebrates (see Section VIII.B). Upon cleavage, the Notch ligand produces the form NICD, which, after phosphorylation, possibly by the kinase GSK3, migrates to the nucleus where it induces the expression of genes like *Hairy* and *Lfng*. The FRINGE protein inhibits the cleavage of Notch into NICD. The model based on this negative feedback regulation shows that it can give rise to sustained oscillations.

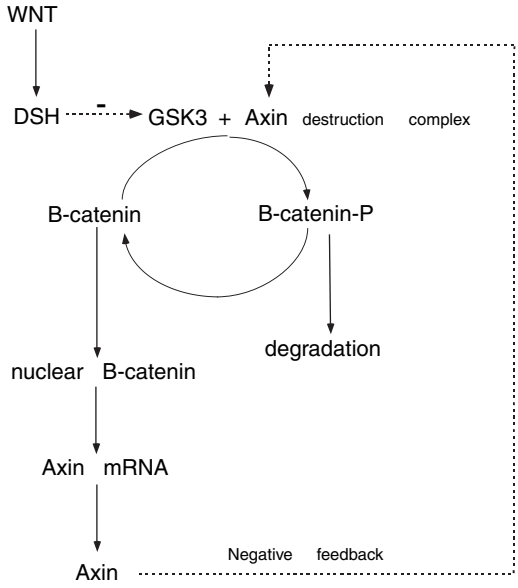

Figure 5. A negative feedback mechanism in the Wnt signaling pathway can also give rise to periodic gene expression. This negative feedback on transcription could form the core of the segmentation clock mechanism by driving oscillations in the Notch pathway. Wnt signaling activates the ligand DSH which inhibits the β-catenin destruction complex formed by the protein Axin and the kinase GSK3. This destruction complex phosphorylates β-catenin and thereby marks it for degradation. Negative feedback originates from the fact that β-catenin induces the expression of the Axin gene; translation of Axin mRNA results in the accumulation of the Axin protein, which leads to β-catenin degradation. Here again a model based on this negative feedback regulation shows that it can produce sustained oscillations. Coupling of the Notch oscillator to the Wnt oscillator could be mediated by oscillations in the activity of the kinase GSK3.

segmentation clock could be based [165]. The possibility of sustained oscillations resulting from such negative feedback loops has been investigated in theoretical models [169, 170] that emphasize the role of time delays in the appearance of sustained oscillations. A role for intercellular signaling through the Notch-Delta pathway has also been pointed out in the model proposed for the zebra fish segmentation clock [169].

One intriguing aspect of the models for the segmentation clock is their link with mechanisms proposed for circadian rhythms. Both types of oscillations are based on negative autoregulation of gene expression. The question arises as to how similar mechanisms produce oscillations with a period in the range 30 min to 2 h for the segmentation clock and around 24 h for circadian rhythms. It would be interesting to further characterize the differences that lead to a 10-fold change in period in the two situations. Such differences may pertain, for

example, to the half-life of proteins or mRNAs or to the post-transcriptional regulation of proteins involved in the oscillatory mechanism.

Oscillations of the segmentation clock with a period of 2 h have also been observed in fibroblast cell cultures following serum shock. There also, oscillations in the expression of the gene *Hes1* related to the Notch pathway have been attributed to negative feedback on transcription [171]. The periodic operation of the segmentation clock was recently demonstrated in cells of the PSM, where intercellular coupling is needed to prevent damping of the oscillations [172].

Progress has also been made on the experimental characterization of the biochemical process that mediates the anterior to posterior progression of the determination wavefront along the PSM during somitogenesis. Fibroblast growth factor (FGF) signaling is involed in the coupling between the segmentation clock and the formation of somites [173]. Moreover, a gradient in *fgf* mRNA, starting at the posterior end of the PSM, extends in the direction of the anterior end where its progressive degradation results in the anterior to posterior movement of the wavefront [174]. The study of a theoretical model has recently shown [175] that the bistability assumed in the clock and wavefront model [160] could originate from the antagonistic gradients of mutually inhibiting FGF and retinoic acid [176] along the PSM. Mutual inhibition is known to give rise to bistability, as demonstrated, for example, in a synthetic genetic network [177].

C. Nucleocytoplasmic Oscillations of the Transcription Factor Msn2 in Yeast

In the yeast *Saccharomyces cerevisiae*, two related transcriptional activators Msn2 and Msn4 are activated in various stress conditions. These proteins are located in the cytoplasm, but they migrate to the nucleus upon activation. Translocation to the nucleus is inhibited by high activity of the cAMP-PKA pathway. Using time-lapse video microscopy on single cells, Jacquet et al. [87] followed the kinetics of translocation of Msn2 fused to the Green fluorescence protein (GFP). They showed that light emission of the microscope is sufficient to induced migration of Msn2 to the nucleus and therefore is sensed as a stress by the cell. Unexpectedly, the population of Msn2 molecules displayed an oscillatory behavior, shuttling repetitively between nucleus and cytoplasm upon light stress, with a periodicity of the order of several minutes. The phenomenon presents a large variability between individual cells. Upon additional stress the oscillatory behavior is maintained but the average time spent in the nucleus is increased. A plausible theoretical model was proposed to account for such oscillations, based on the hypothesis that this transcriptional regulator is involved in an autoregulatory loop controlling its nuclear localization [87].

An alternative possibility is that a biochemical oscillator controls the periodic shuttling of Msn2 between cytosol and nucleus. So far the existence of such a putative biochemical oscillator driving Msn2 oscillatory shuttling has not been substantiated by experimental observations. Among possible biochemical oscillators with periods in the range of minutes, glycolysis could be a natural candidate (see Section IV), but glycolytic oscillations are controlled by glucose rather than stress. Calcium oscillations have not been observed in yeast, and their occurrence may be precluded by the fact that this organism lacks $InsP_3$ receptors. Recent observations [178] suggest that oscillatory shuttling of Msn2 may well be controlled by oscillations of cAMP, via the protein kinase PKA, which is activated by cAMP. It appears indeed that the cellular localization of Msn2 is governed through phosphorylation by PKA.

As shown by a theoretical model [178], the periodic variation of cAMP could originate from the negative feedback exerted via PKA on the synthesis of cAMP (Fig. 6). Thus, PKA could exert its negative control by inactivating through phosphorylation the GAP protein, which is involved in the activation of adenylate cyclase, or by activating the enzyme phosphodiesterase, which degrades cAMP. Such a mechanism producing intracellular oscillations of cAMP could operate in other cell types. Thus it is closely related to the intracellular mechanism proposed for cAMP oscillations in *Dictyostelium* [84, 85]. Oscillations of cAMP were also proposed to underlie pulsatile hormone release in GnRH secreting cells [179]. The evidence for cAMP oscillations in yeast so far remains indirect, since the variations of this metabolite cannot be followed continuously. The oscillatory nucleocytoplasmic shuttling of Msn2 nevertheless provides an indirect sign that cAMP might oscillate in yeast.

IX. COMPLEX OSCILLATORY BEHAVIOR

The transition from simple to complex oscillatory phenomena is often observed in biochemical and cellular systems. Thus, bursting represents one type of complex oscillations that is particularly common in neurobiology [29]. An active phase of spike generation is followed by a quiescent phase, after which a new active phase begins. Mathematical models throw light on the conditions that generate such complex periodic oscillations [180]. Chaos is another common mode of complex oscillatory behavior that has been studied intensively in physical, chemical, and biological systems [31, 122, 181]. These irregular oscillations are characterized by their sensitivity to initial conditions, which accounts for the unpredictable nature of chaotic dynamics.

Yet another type of complex oscillatory behavior involves the coexistence of multiple attractors. Hard excitation refers to the coexistence of a stable steady state and a stable limit cycle—a situation that might occur in the case of circadian rhythm suppression discussed in Section VI. Two stable limit

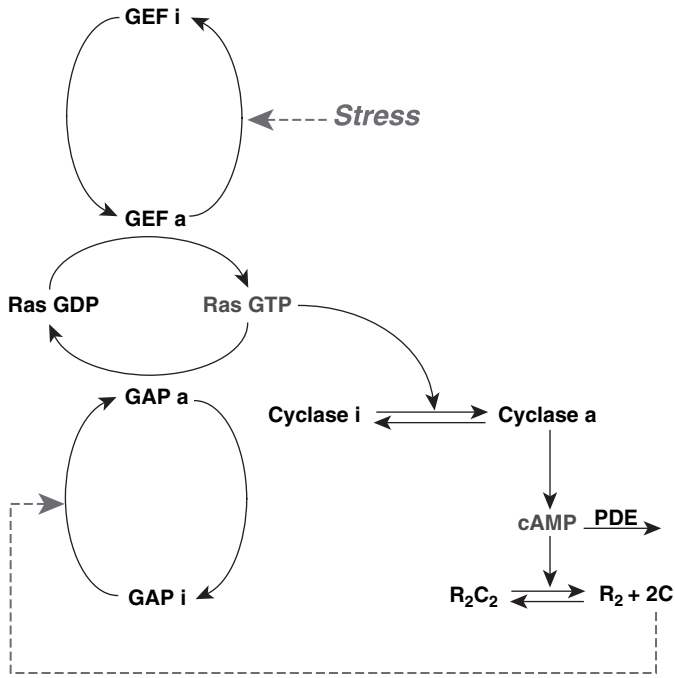

Figure 6. Regulation of the cAMP-PKA pathway in yeast. Synthesis of cAMP by adenylate cyclase is enhanced when this enzyme is activated by Ras-GTP, the active form of the Ras protein. The transformation of the inactive form Ras-GDP into Ras-GTP is triggered by the active form GEFa of the GEF protein, while the reverse, inactivating step is elicited by the active form GAPa of the GAP protein. Protein kinase A is activated when cAMP binds to the holoenzyme form R_2C_2 and frees the active, catalytic subunit C. Negative feedback originates from the fact that the catalytic subunit C of PKA phosphorylates and thereby activates the inactive form GAPi into GAPa. This leads to a decrease in Ras-GTP and, subsequently, to a drop in cAMP. A model based on this negative feedback regulation shows the possibility of sustained oscillations in cAMP and PKA activity [178]. These oscillations could be triggered by the stress-induced inactivation of GEFa. Stress-induced, nucleocytoplasmic oscillations of the transcription factor Msn2 in yeast would originate from the control of its subcellular localization through phosphorylation by the catalytic subunit of PKA (see Section VIII.C).

cycles may also coexist, separated by an unstable limit cycle. This phenomenon, referred to as birhythmicity [182], is the oscillatory counterpart of bistability in which two stable steady states, separated by an unstable state, coexist. Birhythmicity was predicted theoretically before being observed experimentally.

The study of models indicates the existence of two main routes to complex oscillatory phenomena. The first relies on forcing a system that displays simple

periodic oscillations by a periodic input [122]. In an appropriate range of input frequency and amplitude, one can often observe the transition from simple to complex oscillatory behavior such as bursting and chaos. For other frequencies and amplitudes of the forcing, entrainment or quasiperiodic oscillations occur. Because circadian rhythms are naturally subjected to periodic forcing by LD cycles, the possibility arises that such forcing might lead to chaos. Such a situation would be detrimental to the organism, since entrainment by the LD cycle is precisely the most conspicuous function of circadian rhythms. Numerical simulations of a model for the circadian clock indicate that entrainment, quasiperiodic oscillations, and chaos may indeed occur, depending on the magnitude of the periodic changes induced by the LD cycle in the light-sensitive parameter. The waveform of the forcing, however, is also important since the domain of entrainment enlarges at the expense of chaos when the periodic variation in the light-sensitive parameter changes from square wave to sinusoidal [183]. Given that the real LD cycle differs from a square wave, the possibility that chaotic dynamics in the circadian control system seems unlikely in natural conditions.

Complex oscillations can also occur in autonomous systems that operate in a constant environment. The study of models for a variety of cellular oscillations shows that complex oscillatory phenomena may arise through the interplay between several instability-generating mechanisms, each of which is capable of producing sustained oscillations [31, 182]. The case of Ca^{2+} signaling is particularly revealing because of the multiplicity of feedback mechanisms that could potentially be involved in the onset of oscillations. Thus, among the many nonlinear processes that could take part in an instability-generating loop are (1) Ca^{2+}-induced Ca^{2+} release, (2) desensitization of the $InsP_3$ receptor, (3) bell-shaped dependence of the $InsP_3$ receptor on Ca^{2+} that reflects its activation and inhibition at different Ca^{2+} levels, (4) capacitative Ca^{2+} entry, (5) PLC or/and $InsP_3$ 3-kinase activation by Ca^{2+}, (6) control of Ca^{2+} by mitochondria, (7) G-protein regulation by Ca^{2+}, and (8) coupling of the membrane potential to cytosolic Ca^{2+}. Several models in which at least two of these regulatory processes are coupled were shown to admit birhythmicity, bursting, or chaotic oscillations [62, 184–187]. Chaotic dynamics has been observed experimentally in glycolysis in autonomous conditions [188], presumably as a result of the interplay between two endogenous instability-generating mechanisms.

X. CONCLUDING REMARKS

Since the onset of studies on dissipative structures at the end of the 1960s [12, 14, 15, 18, 189], the field devoted to the investigation of nonequilibrium structures in chemistry, physics, and the life sciences has grown tremendously. In this chapter I focused on temporal dissipative structures in biology, a field where they

abound. My aim was to provide an overview of oscillatory processes in biological systems, including recent developments and new examples. Rhythmic phenomena are common at all levels of biological organization because the thermodynamic and kinetic conditions for the occurrence of rhythmic behavior are particularly well-satisfied [15, 18]. Indeed, biological systems are open, since they exchange matter and energy with their surroundings; they often function far from equilibrium; and their evolution is governed by nonlinear kinetic laws.

The sources of nonlinearity are manifold in biological systems. Besides cooperativity, a major source of nonlinearity is provided by the variety of regulatory processes encountered at the cellular level (see Table III). The existence of regulatory feedback is thus the main reason why rhythmic behavior is among the most conspicuous properties of living organisms. Positive feedback is generally associated with multiple steady states [30], while negative feedback is capable of generating oscillations provided a minimum delay in the negative feedback loop exists. Inhibition by the product is indeed at the core of many rhythmic phenomena in biological systems. To name but a few among those discussed in this chapter, circadian rhythms, oscillations in NF-KB or p53, and the segmentation clock all appear to be based on direct or indirect, negative autoregulation. Negative feedback is also involved in the *Repressilator*, which is a synthetic oscillatory gene circuit consisting of three repressors coupled in a cyclical manner [26, 190]. Beyond the particular nature of the molecules involved, mathematical models help to establish links between various oscillatory mechanisms based on inhibitory feedback [170].

In oscillatory mechanisms based on negative feedback, a positive effect is generally needed to sustain periodic behavior, but it can just be an induction process—as is the case for the action of CLOCK/BMAL1 in the circadian clock—and does not need to take the form of positive feedback. Positive feedback is self-amplifying and amounts to activation by the product. Such mode of regulation is less common than negative feedback but plays a key role in the origin of several biological rhythms. Besides oscillations of the membrane potential in nerve and muscle cells, which were not considered in detail in this chapter devoted to cellular rhythms of nonelectrical nature, examples of rhythms based on positive feedback include glycolytic oscillations, Ca^{2+} spiking, and cAMP oscillations in *Dictyostelium* cells.

To give rise to oscillatory behavior instead of a biochemical explosion, self-amplification must, however, be coupled to a limiting process. Such a limiting process can be viewed as a form of negative feedback because it occurs as a consequence of the positive feedback that precedes it. Thus, in the case of glycolytic oscillations, the activation of phosphofructokinase by a reaction product is followed by a counteracting fall in the rate of the enzymatic reaction, due to the enhanced substrate consumption associated with enzyme activation. In Ca^{2+} pulsatile signaling, the explosive rise in cytosolic Ca^{2+} due

to Ca^{2+}-induced Ca^{2+} release from the endoplasmic reticulum is limited by the emptying of the store and by the inhibition of the Ca^{2+} channel at high levels of cytosolic Ca^{2+}. Likewise, in *Dictyostelium*, the rapid rise in cAMP due to positive feedback necessarily leads to its subsequent decrease through cAMP-induced desensitization of the cAMP receptor on the plasma membrane.

Sometimes a mixture of positive and negative feedback can produce relaxation oscillations based on bistability. This situation is illustrated by the oscillations in cdk1 activity, which drive the early cell division cycles in amphibian embryos (see Section VII). While oscillations may originate from the negative feedback exerted by the cyclin-dependent kinase cdk1 (cdc2) through activation of the cyclin degradation pathway [133], bistability has been shown to arise from positive feedback of cdk1 through activation of phosphatase cdc25 and inhibition of kinase wee1, which respectively activate and inhibit cdk1. This bistability, coupled to the negative feedback loop, results in repetitive cycles of hysteresis, which correspond to robust, sustained oscillations of cdk1 activity [135–137].

A striking property that is well illustrated by recently discovered examples of rhythms in genetic networks is that multiple oscillatory mechanisms can coexist in a given biological system. This is exemplified by the case of circadian rhythms discussed in Section VI and by the vertebrate segmentation clock considered in Section VIII. In the latter system, several negative feedback loops are present in the Notch or Wnt signaling pathways, providing for multiple potential sources of oscillatory behavior [165]. The reasons for such a multiplicity of oscillatory mechanisms may be manifold. One may be to provide redundancy so that back-up mechanisms are available in case of failure of one of the regulatory loops. That a feedback loop exists does not guarantee, however, that it operates in the parameter domain producing sustained oscillations. Even when multiple feedback loops are present within a system, some of them may mot be capable of producing oscillations by themselves because the values of the parameters that characterize the feedback module correspond to the evolution toward a stable steady state. Another reason for the multiplicity of instability-generating mechanisms may simply be to strengthen the oscillations by enlarging the domain of rhythmic behavior in parameter space.

As recalled in Section IX, models indicate that the interplay between several endogenous mechanisms may give rise to complex oscillations in the form of bursting or chaos. The multiplicity of oscillatory mechanisms within a given metabolic or genetic network may therefore bear, positively—as in the case of bursting oscillations or, adversely, as perhaps in the case of chaos—on the physiological functions of cellular rhythms. Even in the presence of interactions between multiple instability-generating mechanisms, however, simple periodic behavior or oscillations of the bursting type remain more common than chaos in parameter space.

In several instances, intracellular oscillations are linked to the formation of spatial or spatiotemporal patterns, as shown by the role of the vertebrate segmentation clock in somitogenesis, by the propagation of intra- or intercellular waves in Ca^{2+} signaling in many cell types, and by cAMP signaling in *Dictyostelium* amoebae. Recent observations on the occurrence of intracellular waves in activated leukocytes [191] provide yet another example of close link between temporal and spatial organization at the cellular level. The close intertwining of spatial and temporal patterns led Duboule [192] to write that ''animal development is, in fact, nothing but time.''

Since the initial impetus given by Ilya Prigogine to the study of oscillatory phenomena in chemical and biological systems, the number of examples of periodic behavior has grown immensely. While some examples were known for long, new ones were added to the list, which will undoubtedly continue to expand at an increasing pace. The views of Ilya Prigogine on nonequilibrium self-organization in the form of dissipative structures provide a conceptual framework that allows us to unify the multifarious rhythms that occur in biological systems with periods spanning more than 10 orders of magnitude. This global perspective underlines the links between rhythmic phenomena occurring in widely different biological settings, from genetic to metabolic and neural networks and from cell to animal populations.

Acknowledgments

I wish to dedicate this chapter to the memory of Ilya Prigogine, who triggered my interest in the modeling of rhythmic phenomena in biological systems and deeply influenced this research by the originality of his views and his unwavering enthusiasm and generosity. To this homage I wish to associate the name of Benno Hess, who did much to extend the study of nonequilibrium self-organization phenomena to the field of biological sciences. Thanks are due to members of my research group for fruitful discussions. This work was supported by grant #3.4636.04 from the *Fonds de la Recherche Scientifique Médicale* (F.R.S.M., Belgium) and by the European Union through the Network of Excellence BioSim, Contract No. LSHB-CT-2004-005137. The chapter was prepared while the author held a *Chaire Internationale de Recherche Blaise Pascal de l'Etat et de la Région Ile-de-France, gérée par la Fondation de l'Ecole Normale Supérieure* in the Institute of Genetics and Microbiology at the University of Paris-Sud 11 (Orsay, France).

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