Computational approaches to cellular rhythms

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Oscillations arise in genetic and metabolic networks as a result of various modes of cellular regulation. In view of the large number of variables involved and of the complexity of feedback processes that generate oscillations, mathematical models and numerical simulations are needed to fully grasp the molecular mechanisms and functions of biological rhythms. Models are also necessary to comprehend the transition from simple to complex oscillatory behaviour and to delineate the conditions under which they arise. Examples ranging from calcium oscillations to pulsatile intercellular communication and circadian rhythms illustrate how computational biology contributes to clarify the molecular and dynamical bases of cellular rhythms.

Rhythmic phenomena represent one of the most striking manifestations of dynamic behaviour in biological systems. In 1936, Fessard[1] published a book on the *Rhythmic Properties of Living Matter*. This book was devoted solely to the oscillatory properties of nerve cells, but it is now clear that rhythms are encountered at all levels of biological organization, with periods ranging from a fraction of a second to years[2,3]. These rhythms find their roots in the many regulatory mechanisms that control the dynamics of living systems. Thus, at the cellular level, neural and cardiac rhythms are associated with the regulation of voltage-dependent ion channels, metabolic oscillations originate from the regulation of enzyme activity, pulsatile intercellular signals and intracellular calcium oscillations involve the control of receptor activity or transport processes, while regulation of gene expression underlies circadian rhythms.

Understanding the molecular and cellular mechanisms responsible for oscillations is crucial for unravelling the dynamics of life. When based firmly on experiments, computational biology provides an essential tool for studying these mechanisms which, because of their complexity, cannot be comprehended by sheer intuition alone. The purpose of this article is to present an overview of how models and computer simulations are used to address the origin, properties and functions of some of the main cellular rhythms.

Theoretical models for biological rhythms were first used in ecology to study the oscillations resulting from interactions between populations of predators and prey[4]. Neural rhythms represent another field where such models were used at an early stage: the formalism developed by Hodgkin and Huxley[5] still forms the core of most models for oscillations of the membrane potential in nerve and cardiac cells[6-8]. Of more recent vintage are models for oscillations of non-electrical nature that arise at the cellular level from regulation of enzyme, receptor or gene activity (see ref. 3 for a detailed list of references).

The computational biology of these rhythms forms the core of this review. I shall consider, in turn, oscillations of intracellular calcium, pulsatile signalling in intercellular communication, and circadian rhythms. Additionally, I shall describe how computational biology can help in understanding the transition from simple periodic behaviour to complex oscillations including bursting and chaos.

Basic phenomenology of oscillatory phenomena

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[9]. Feedback processes and cooperativity are two main sources of nonlinearity that favour the occurrence of instabilities in biological systems. When the steady state becomes unstable, the system moves away from it, often bursting into sustained oscillations around the unstable steady state (Fig. 1a, b).

In the phase space defined by the system's variables (for example, the concentrations of the biochemical species that are involved in the oscillatory mechanism), sustained oscillations correspond to the evolution towards a closed curve — the limit cycle. These oscillations are resistant to perturbations, because the limit cycle will be regained regardless of initial conditions, starting from the vicinity of the unstable state (Fig. 1c) or from outside the asymptotic, closed trajectory (Fig. 1d). Limit-cycle oscillations thus represent an example of non-equilibrium self-organization and can therefore be viewed as temporal dissipative structures[9]. The oscillations are characterized by their amplitude and by their period. A bifurcation diagram can be constructed by plotting the amplitude of the oscillations of a given variable and the steady state (stable or unstable) as a function of a control parameter (see Box 1).

Evolution towards a limit cycle is not the only possible behaviour when a steady state becomes unstable in a spatially homogeneous system. The system may evolve towards 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 be important in differentiation[10], and was shown recently to have a role in early *Xenopus* development[11]. When spatial inhomogeneities develop, instabilities may lead to the emergence of spatial or spatiotemporal dissipative structures[9]. These can take the form of propagating concentration waves, which are closely related to oscillations.

Figure 1 Sustained oscillations can occur in models based on positive or negative feedback. **a**, Typical oscillations obtained in models based on positive feedback. The particular oscillations shown are obtained in a two–variable model for the product-activated phosphofructokinase reaction responsible for glycolytic oscillations[3,81]. Y represents the reaction product, and X represents the substrate of the enzyme. Similar oscillations are obtained in models for Ca^{2+} oscillations based on Ca^{2+}-induced Ca^{2+} release[3,17] or cAMP oscillations in *Dictyostelium* amoebae[3,28]. In the case of Ca^{2+} oscillations, Y denotes cytosolic Ca^{2+}, whereas X represents the Ca^{2+} content of intracellular stores. For cAMP oscillations in *Dictyostelium*, which rely on a mixture of positive and negative feedback (see text), X represents the fraction of active (non-desensitized) cAMP receptor, and Y represents the level of extracellular cAMP. **b**, Oscillations obtained in a five-variable model based on negative feedback for the circadian rhythmic variation of the PER protein (Y) and its mRNA (X) in *Drosophila*[3,50]. **c**, Limit cycle in the phase plane (X, Y), corresponding to the oscillations shown in **a**. Initial conditions are such that the limit cycle here is reached from a point located in the vicinity of the unstable steady state. **d**, Limit cycle corresponding to the oscillations shown in **b**. Initial conditions are such that the limit cycle

here is reached from outside. The arrows on the phase plane trajectory in **c** and **d** indicate the direction of movement along the limit cycle. Over a period, oscillations in **a** and **b** can be broken down into phases 1–3 and 1–2, respectively (see text and below). As in Fig. 3, actual scales for variables X and Y and governing kinetic equations are given in the original references indicated in the figure legends. Curves were obtained by numerical integration of these equations, using the Berkeley Madonna software. In both **a** and **b**, the rise in Y, brought about by the rise in X, leads to a drop in X. This drop is followed by a decrease in Y that eventually allows for the next rise in X. Thus, although the regulatory interactions are of opposite nature, the phase relationship between the variables is similar. One feature, however, differs between the two situations. In the case of positive feedback (**a**), the time lag associated with phase 3 results in the pulsatile nature of the oscillations: sharp peaks, or spikes, are generated at regular
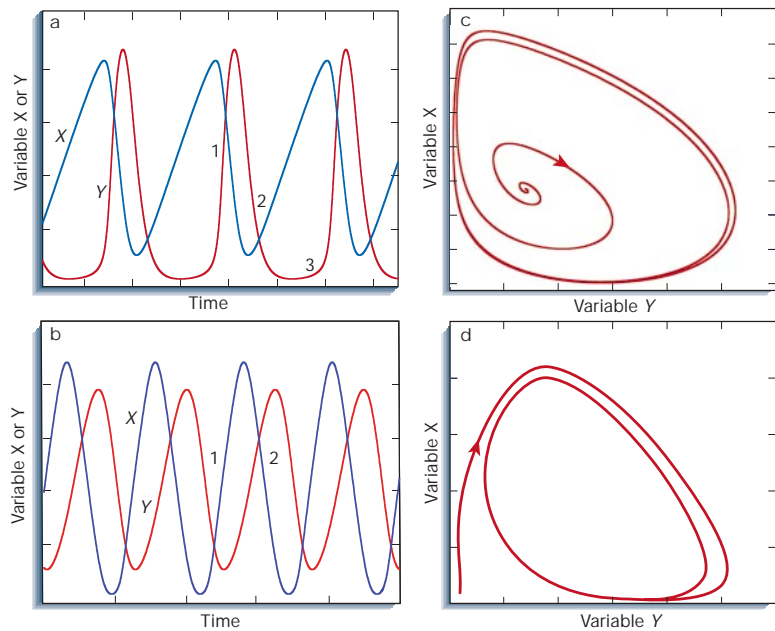
intervals. Phase 3 corresponds to the pacemaker potential that brings electrically excitable cells up to the depolarization threshold beyond which an action potential is generated. This pulsatility is generally not seen in models based on negative feedback (**b**), which lack phase 3. However, negative feedback can also produce spikes at regular intervals when the oscillatory mechanism involves the passage through thresholds (such a situation producing oscillations resembling those of **a** is encountered in a model of a phosphorylation cascade for the mitotic oscillator in amphibian embryonic cells[3,72]). In both **a** and **b**, the driving force behind sustained oscillations is found in the phase of increase of variable X, which does not require any positive feedback. In the different examples considered here, it suffices that the rise in X is brought about by some constitutive process such as substrate replenishment, receptor resensitization, refilling of Ca^{2+} stores or gene transcription.

Understanding the molecular mechanism of oscillations requires clarifying the chain of events that cause each variable of the system to periodically rise and fall. Elucidation of the underlying mechanism largely reduces to identifying the feedback processes that lie at the core of the oscillations. The latter may originate from positive (Fig. 1a) or negative (Fig. 1b) 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 behaviour without resorting to modelling and computer simulations.

In addressing the molecular mechanism of a biological rhythm, the typical programme of computational biology consists of the following steps. First, the key variables of the phenomenon are identified, together with the nature of their interactions that form the relevant feedback loops. Second, differential equations describing the time evolution of the system are constructed. In spatially homogeneous conditions, these take the form of ordinary differential equations, whereas in the presence of diffusion, partial differential equations are used to describe the system's spatiotemporal evolution. Third, the steady state(s) admitted by these equations are determined analytically or by numerical integration.

The fourth step probes the stability properties of the steady state(s). This is generally done by using linear stability analysis. The principle of this analysis[9] is to determine the evolution of infinitesimal perturbations away from the steady state: the steady state is stable when such perturbations decay in time, and unstable otherwise. When parameter values correspond to an unstable steady state,

numerical integration of the evolution equations should confirm that in the course of time the system leaves the steady state to evolve either to another, stable steady state or to sustained limit-cycle oscillations.

Using this approach, the fifth step is to determine the domains of occurrence of sustained oscillations in parameter space. Numerical solution of the kinetic equations then allows the construction of bifurcation diagrams that show how the period and amplitude vary as a function of the various parameters. Bifurcation diagrams may also be generated by means of programs (such as AUTO, developed by Doedel[12]) which are based on continuation methods. Finally, the theoretical predictions of the model, obtained by numerical simulations based on available parameter values, or else on values taken in a physiological range, are compared with experimental observations. This programme possesses its own dynamics: when the model predictions do not agree with experiments, or when new behaviours are discovered, the model must be modified accordingly.

Calcium oscillations

The three best-known examples of biochemical oscillations were found during the decade 1965 to 1975[3,13,14]. These include the peroxidase reaction, glycolytic oscillations in yeast and muscle, and the pulsatile release of cyclic AMP (cAMP) signals in *Dictyostelium* amoebae (see below). 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[15]. These oscillations are often accompanied by propagation of intracellular or intercellular Ca^{2+} waves. The significance of Ca^{2+} oscillations and waves stems from the crucial importance of this ion in the control of many key cellular processes[15].

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 then 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[15]. A conspicuous feature of Ca^{2+} release is that it is self-amplified: cytoplasmic Ca^{2+} triggers the release of Ca^{2+} from intracellular stores, a process known as Ca^{2+}-induced Ca^{2+} release (CICR).

A first model for cytosolic Ca^{2+} oscillations was based[16] on the activation of PLC by Ca^{2+}. Although this positive feedback regulation has been observed in some cell types, it seems that a more general feedback process underlying oscillations is CICR itself. The effect of CICR positive feedback is antagonized by several regulatory processes (see below). A simple two-variable model for signal-induced Ca^{2+} oscillations based on CICR accounts[17] for sustained oscillations of cytosolic Ca^{2+}. These oscillations occur between two critical values of the stimulus intensity, for example, two critical levels of the hormonal signal (see figure in Box 1). Below the lower critical value, a low steady-state level of cytosolic Ca^{2+} is established; above the larger critical value, the system evolves towards a higher, stable steady-state level 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[18], but still attribute to CICR a primary role in the origin of repetitive Ca^{2+} spiking.

Mathematical models for Ca^{2+} signalling have developed in two additional directions. First, waves of intra- or intercellular Ca^{2+} have been modelled by incorporating the diffusion of cytosolic Ca^{2+} or the passage of Ca^{2+} or $InsP_3$ from cell to cell through gap junctions[19–22]. Although 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[15,23]. Second, computational biology enables one 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[24]. Among the processes that could underlie such frequency encoding are protein (de)phosphorylation by a Ca^{2+}-dependent kinase (phosphatase)[17], or the Ca^{2+}-dependence of calmodulin-kinase II[25]. A recent study combining experimental and modelling approaches showed the possibility of frequency encoding of Ca^{2+} spikes by interplay with cAMP signalling[26].

Pulsatile signalling in intercellular communication

Although intracellular information can be encoded in the frequency of signal-induced Ca^{2+} spikes, some extracellular signals can themselves be produced in a pulsatile manner. Examples of pulsatile intercellular communication include episodic hormone secretion and pulsatile signals of cAMP in the slime mould *Dictyostelium discoideum*. After starvation, these amoebae undergo a transition from a unicellular to a multicellular phase of their life cycle. By a chemotactic response to cAMP signals, as many as 10^5 amoebae collect around cells behaving as aggregation centres. These centres release cAMP with a period of about 5 minutes; surrounding cells relay the chemotactic signal towards the periphery of the aggregation field. Relay and oscillations of cAMP result in the formation of concentric or spiral waves of aggregating cells[27].

Models help to clarify the mechanism of cAMP oscillations in *Dictyostelium*[28,29], which involves both positive and negative feedback.

Binding of extracellular cAMP to a cell-surface receptor leads to the activation of adenylate cyclase, which catalyses the synthesis of intracellular cAMP. Transport of cAMP into the extracellular medium creates a positive feedback loop that drives a rapid rise in cAMP synthesis (phase 1 in Fig. 1a). 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 (phase 2). Models confirm[28] that negative feedback attributable to cAMP-induced receptor desensitization through reversible phosphorylation can have such a role in limiting self-amplification. Once the levels of intra- and extracellular cAMP are sufficiently low, dephosphorylation can resensitize the receptor. The ensuing build-up of extracellular cAMP (phase 3) 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 behaviour, 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[28,29]. Thus, relay and oscillations of cAMP are produced by a unique mechanism in adjacent domains in parameter space. The two types of dynamic behaviour are analogous to the excitable or pacemaker behaviour of nerve cells.

Theoretical models shed light on additional aspects of pulsatile cAMP signalling in *Dictyostelium*. First, like Ca^{2+} spikes, cAMP pulses are frequency encoded. Only pulses delivered at 5-min intervals are capable of accelerating slime-mould development after starvation. Simulations indicate that frequency encoding is based on reversible receptor desensitization[28]. The kinetics of receptor resensitization dictates the interval between successive pulses required for a maximum relay response.

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 behaviour, from no relay to relay, and finally to oscillations[3].

Third, models are being used to probe the mechanisms underlying the formation of concentric or spiral waves of cAMP responsible for the spatiotemporal patterns observed during aggregation. Among the factors shown to be important in the transition between the two types of waves are extracellular phosphodiesterase activity[30] and desynchronization of cells that follow the developmental path after starvation[31]. Models based on the same feedback mechanism also account for the propagation of planar and scroll waves within the multicellular slug formed by the amoebae after aggregation[32].

Pulsatile cAMP signalling 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[33] and that the temporal pattern of a hormone is often as important as its concentration in the blood[34]. The best examples of pulsatile hormone signals are those of gonadotropin-releasing hormone (GnRH) secreted by the hypothalamus with a periodicity of 1 h in humans and rhesus monkey[35], growth hormone (GH) secreted with a period of 3 to 5 h[36], and insulin secreted by pancreatic β-cells with a period close to 13 min in humans[37]. 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[35,36].

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 it does for cAMP pulses in *Dictyostelium*[38,39]. The mechanism of GnRH pulsatility is still unknown and provides a challenge for both experiments

Box 1
Bifurcation diagrams

Plotting the maximum and minimum of the oscillations of a given variable as a function of a control parameter allows the construction of a 'bifurcation diagram' showing where the system changes its dynamical properties. A common type of bifurcation diagram is depicted in the figure opposite for the case of intracellular Ca^{2+} oscillations. Below a critical value, the system reaches a stable steady state, while at the critical value the steady state becomes unstable and a stable limit cycle begins to grow, surrounding the unstable steady state (the critical value at which the limit cycle appears corresponds to a Hopf bifurcation). The amplitude of the limit cycle increases and passes through a maximum as the value of the control parameter increases. Finally, above a second, higher critical value, sustained oscillations disappear and the system again evolves towards a new stable steady state. This bifurcation diagram illustrates an important property of sustained oscillations, namely that they occur within a certain parameter range often bounded by two critical values.

The scheme in figure opposite represents the simplest type of bifurcation diagram for the onset of sustained oscillations. More complex bifurcation diagrams are obtained when multiple attractors (that is, stable steady states or stable oscillations) coexist in a certain range of parameter values. Thus a stable steady state and a stable limit cycle, corresponding to sustained oscillations, may coexist, separated by an unstable limit cycle. Such a situation is referred to as hard excitation. Other modes of attractor multiplicity include the coexistence between two stable steady states (bistability) or between two stable limit cycles (birhythmicity). Each of the stable attractors possesses its basin of attraction, which includes all initial conditions, in phase space, from which the system evolves towards this particular attractor.
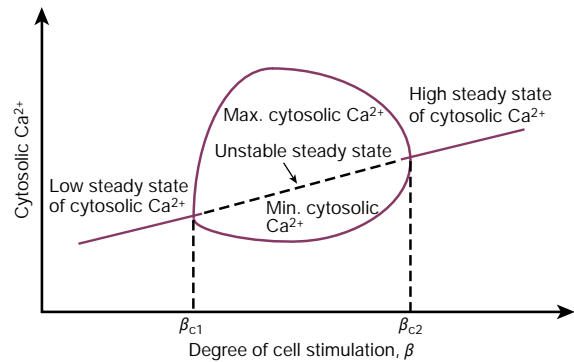
Box 1 Figure Schematic bifurcation diagram showing the domain and amplitude of intracellular Ca^{2+} oscillations as a function of the degree of external stimulation β, which is used as control parameter. Sustained Ca^{2+} oscillations occur in a range of stimulation between the two critical β-values 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. In the situation described, a unique steady state corresponds to a given value of β; the precise value of the steady state depends on β. The bifurcation diagram is obtained in a two-variable model[17] for Ca^{2+} oscillations based on Ca^{2+}-induced Ca^{2+} release (see ref. 17 for a nonschematic version of the diagram).

and theory. The basis of pulsatile GH secretion has been studied by a modelling approach[40]. In β-cells, pulsatile insulin release could originate from insulin feedback on glucose transport into the cells[41] or from oscillatory membrane activity driven by glycolytic oscillations[37]. Together with these 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.

Circadian rhythms

The most ubiquitous biological rhythms are those that occur with a period close to 24 h and that allow organisms to adapt to periodic variations in the terrestrial environment. Experimental advances during the past decade have clarified the molecular bases of these circadian rhythms, first in *Drosophila* and *Neurospora*, and more recently in cyanobacteria, plants and mammals[42–44]. In 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[45].

Before details on the molecular mechanism of circadian rhythms began to be uncovered, theoretical models borrowed from physics were used to investigate their dynamic properties. 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 modelling the response of human circadian oscillations to light[46] and to account for experimental observations on increased fitness due to resonance of the circadian clock with the external light–dark cycle in cyanobacteria[47]. The earliest model predicting oscillations due to negative feedback on gene expression was proposed by Goodwin[48], at a time when the part played by such a regulatory mechanism in the origin of circadian rhythms was not yet known. Models of this type are still being used in studies of circadian oscillations, for example in *Neurospora*[49].

Molecular models for circadian rhythms were proposed[50] initially for circadian oscillations of the period (PER) protein and its mRNA in

Drosophila, the first organism for which detailed information on the oscillatory mechanism became available[45] (the PER protein behaves as a transcriptional regulator capable of influencing the expression of a variety of genes besides its own gene, *per*). 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[50] governed by a set of five kinetic equations is shown in Fig. 2a; it is based on the negative control exerted by the PER protein on the expression of *per*. Numerical simulations show that for appropriate parameter values, the steady state becomes unstable and limit-cycle oscillations appear (Fig. 1b, d).

This early model did not account for the effect of light on the circadian system. Experiments subsequently showed that a second protein, timeless or TIM, forms a complex with PER, and that light acts by inducing TIM degradation[43]. An extended, ten-variable model was then proposed[51], in which the negative regulation is exerted by the PER–TIM complex (Fig. 2b). This model produces essentially the same result, sustained oscillations in continuous darkness. In addition, it accounts for the behaviour 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 cycles and for the phase shifts induced by light pulses[51]. A closely related model incorporating the formation of a PER–TIM complex has been proposed for *Drosophila* circadian rhythms[52].

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[44]. However, in mammals, the role of TIM as a partner for PER is played by the cryptochrome (CRY) protein, and light acts by inducing gene expression rather than protein degradation as in *Drosophila*[44]. A further analogy between *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
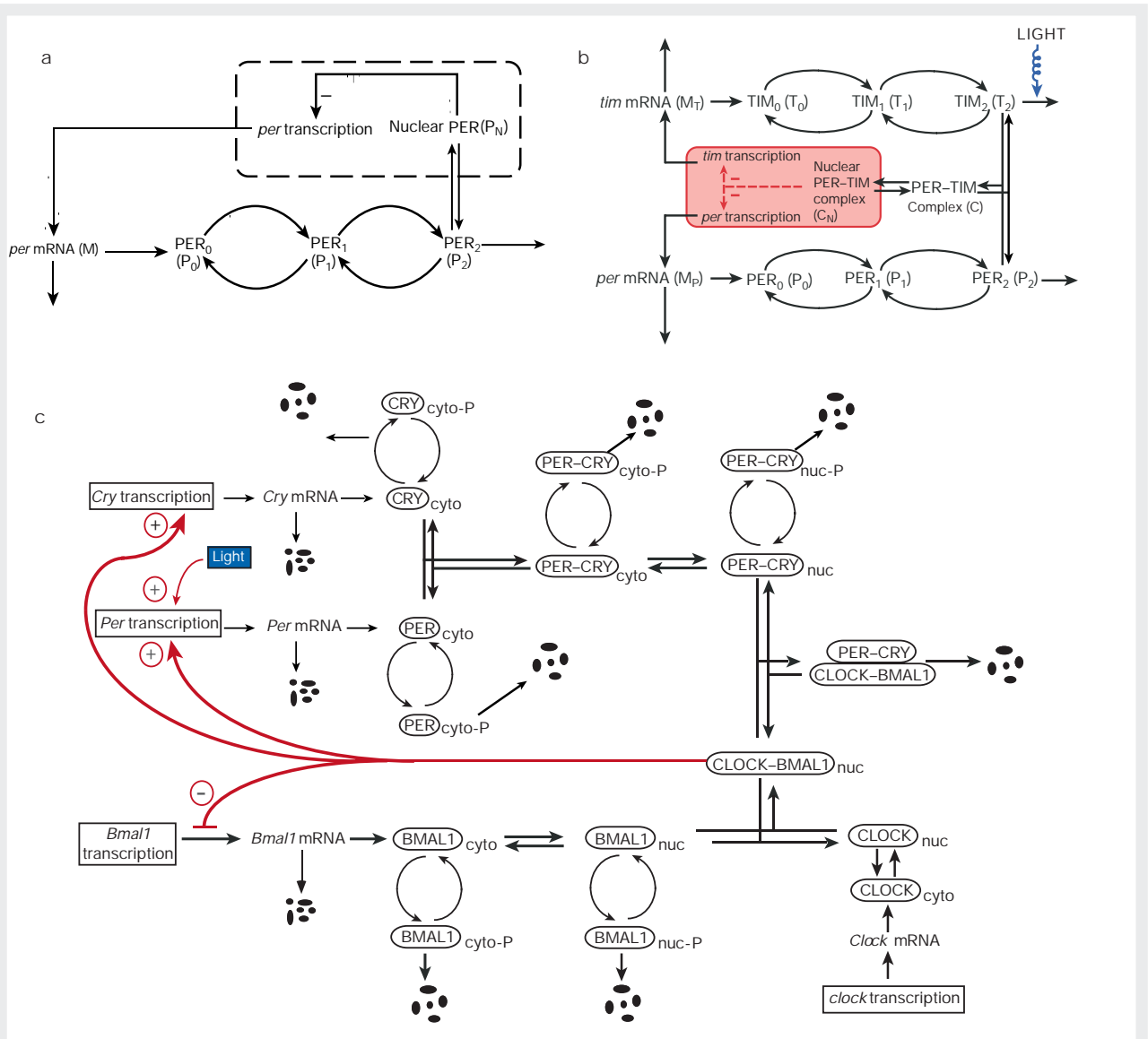
Figure 2 Molecular models of increasing complexity considered for circadian oscillations. **a**, Model for circadian oscillations in *Drosophila* based on negative autoregulation of the *per* gene by its protein product PER[3,50]. The model incorporates gene transcription into *per* mRNA, transport of *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, as well as 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[3,50]. **b**, Model for circadian oscillations in *Drosophila* incorporating the formation of a complex between the PER and TIM proteins[51]. The model is described by a set of ten kinetic equations[51]. **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[58]. 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 the models in **b** and **c** by incorporating light-induced TIM degradation or light-induced *Per* expression, respectively. Further extensions of the model shown in **c** for the mammalian clock are needed to incorporate the recently discovered role of the *Rev-erbα* and *Dec* genes[55,89].

proteins, CLOCK–CYC or CLOCK–BMAL1 in the fly[53] and in mammals[54], 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, which are mediated by other gene products[44,55], are also removed upon formation of the complex with the PER–TIM or PER–CRY dimers[53,54].

What are the dynamical consequences of these additional regulatory loops and of the indirect path of the negative feedback on gene

expression? Addressing these issues requires further extension of the model. Such an extended model has been proposed for *Drosophila*[56,57] and is currently being studied for mammals[58]. The model for the circadian clock mechanism in mammals is schematized in Fig. 2c. The presence of additional mRNA and protein species, as well as of multiple complexes formed between the various clock proteins, complicates the model. The time evolution of this extended model is governed by a system of 16 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
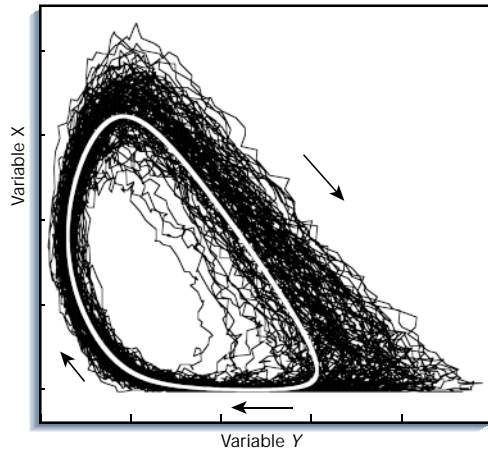
Figure 3 Effect of molecular noise on circadian oscillations. Stochastic simulations of the model of Fig. 2a yield oscillations that correspond, in the phase plane (X, Y), to the evolution to a noisy limit cycle. The latter takes the form of a cloud of points surrounding the deterministic limit cycle (white solid curve) obtained in the absence of molecular noise (data redrawn from Fig. 3a in ref. 64). Variables X (*per* mRNA) and Y (nuclear form of the PER protein) are expressed as numbers of molecules or concentrations in stochastic and deterministic simulations, respectively. In the stochastic simulations illustrated, X and Y vary in the range between 0 and 200 molecules and 20 and 800 molecules, respectively[64]. Arrows indicate the direction of movement along the deterministic or stochastic limit cycle.

mammalian clock, *Bmal1* mRNA oscillates in opposite phase with respect to *Per* and *Cry* mRNAs[44]. Entrainment by the external light–dark cycle can be captured by the model if it incorporates the light-induced increase in the rate of *Per* expression. Numerical simulations show that, upon entrainment, a slight change in parameters, such as the maximum rate of PER phosphorylation, suffices to shift the peak in *Per* mRNA with respect to the onset of the light phase by several hours. This lability could explain why the phase of circadian oscillations in mammals varies in peripheral tissues with respect to the phase of the central pacemaker located in the suprachiasmatic nuclei within the hypothalamus[44].

The results obtained with the model for the mammalian circadian clock provide cues for circadian rhythm sleep disorders in humans[59]. Thus permanent phase shifts in light–dark conditions could account for the familial advanced sleep phase syndrome that has been attributed to PER hypophosphorylation[60], and for the delayed sleep phase syndrome, which is also related to PER[61]. For some parameter values the model fails to allow entrainment by 24-h light–dark cycles. This result could account for the non-24-h sleep–wake syndrome in which the phase of the sleep–wake pattern varies continuously with respect to the light–dark cycle, that is, the patient free-runs in light–dark conditions[59].

Computational biology can provide surprisingly counterintuitive insights. A case in point is the puzzling observation that circadian rhythms in continuous darkness can sometimes be suppressed by a single pulse of light and restored by a second such pulse. Winfree[2] proposed the first theoretical explanation for this long-term suppression. He hypothesized 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 the population would be spread out over the entire cycle so that the cells would be desynchronized and no global rhythm would be seen.

An alternative explanation is based on the coexistence of sustained oscillations with a stable steady state. Such coexistence has been observed[62], albeit in a restricted domain in parameter space, in the model for circadian rhythms in *Drosophila* based on negative autoregulation by the PER–TIM complex (Fig. 2b). 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 limit cycle's basin of attraction corresponding to circadian oscillations[62]. Without a computational model it is impossible to predict the coexistence between a stable steady state and a stable rhythm.

I have discussed only deterministic models for cellular rhythms so far. Do the models remain valid when the numbers of molecules involved are small, as may occur in cellular conditions? 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[63]. The way to assess the influence of molecular noise is to resort to stochastic simulations (see review in this issue by Rao and colleagues, pages 231–237). In applying this approach to the models for circadian rhythms schematized in Fig. 2a, b, we must first break down the different reactions into elementary steps.

The temporal dynamics of the system is determined numerically by allowing the various reaction steps to occur randomly, with a frequency measured by their probability of occurrence. These stochastic simulations show that the dynamic behaviour 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[64]. The larger the numbers of molecules, the smaller the noise due to random fluctuations. In the presence of molecular noise, the trajectory in the phase space transforms into a cloud of points surrounding the deterministic limit cycle (Fig. 3). Stochastic simulations confirm the existence of bifurcation values of the control parameters bounding a domain in which sustained oscillations occur[64]. 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. Mechanisms enhancing resistance to noise in genetic oscillators have been investigated in a recent theoretical study[65].

From simple to complex oscillatory behaviour

Computational biology clarifies the mechanisms responsible for the transition from simple to complex oscillatory phenomena in biochemical and cellular systems[3,66]. Bursting represents one type of complex oscillations that is particularly common in neurobiology. 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 these complex periodic oscillations[67]. Chaos is a common mode of complex oscillatory behaviour that has been studied intensively in physical, chemical and biological systems[3,68,69]. In phase space, chaotic oscillations correspond to the evolution towards a so-called strange attractor. 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 behaviour involves the coexistence of multiple attractors. When a stable steady state and a stable limit cycle coexist (as in the case of suppression of circadian rhythm discussed above), they conspire to produce what is called hard excitation. Two stable limit cycles may also coexist, separated by an unstable cycle. This phenomenon, referred to as birhythmicity[3,66], is the oscillatory counterpart of bistability in which two stable steady states, separated by an unstable state, coexist. Birhythmicity was predicted by numerical simulations before being observed experimentally[3].

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[69]. In an appropriate range of input frequency and amplitude, one can often observe the transition from simple to complex oscillatory behaviour such as bursting and chaos. For other frequencies and amplitudes of

the forcing, entrainment or quasiperiodic oscillations occur. Circadian rhythms are subjected to periodic forcing naturally by light–dark cycles, and numerical simulations of a model for the circadian clock indicate that entrainment, quasiperiodic oscillations and chaos may occur, depending on the magnitude of the periodic changes induced by the light–dark cycle in the light-sensitive parameter[66]. The waveform of the forcing is also important, as the domain of entrainment enlarges at the expense of chaos when the input transforms from a square wave into a sinusoidal forcing[66].

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[3,66]. The case of Ca^{2+} signalling 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+}, which 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[22,66,70,71].

Concluding remarks

Given the rapid accumulation of new data on gene, protein and cellular networks, it is increasingly clear that computational biology will be crucial in making sense of the puzzle of cellular regulatory interactions. Models and simulations are particularly valuable for exploring the dynamic phenomena associated with these regulations. Such an approach has long been applied to the study of biological rhythms, from the periodic activity of nerve and cardiac cells to population oscillations in ecology. I have focused here on the computational biology of some of the main oscillatory phenomena that arise at the cellular level. Additional examples of cellular oscillatory processes that have been studied by means of theoretical models abound. A most important one is the eukaryotic cell cycle. Models indicate that mitosis in amphibian embryonic cells is driven by a limit-cycle oscillator that produces the repetitive activation of the cyclin-dependent kinase cdk1 (refs 72, 73). The interplay between oscillations and bistability has been addressed in detailed molecular models for the cell cycles of yeast and somatic cells, which are more complex owing to the existence of checkpoints[74,75].

At the genetic level, models show that regulatory interactions between genes can result in multiple steady states or oscillations. The two types of phenomena have recently been demonstrated in synthetic genetic networks reconstituted in bacteria (refs 76, 77; and see review in this issue by Hasty and colleagues, pages 224–230). Circadian rhythms are a fertile field for applying computational biology to the study of oscillations associated with genetic regulation. Also related to the regulation of gene expression are the oscillations in the activity of the tumour suppressor p53, which have been studied both experimentally and by means of a model[78]. A segmentation clock involving Notch signalling is responsible for periodic somite formation[79]. Oscillatory nucleocytoplasmic shuttling of the Msn2 transcription factor, with a period of several minutes, has recently been observed in yeast and studied theoretically[80].

Glycolytic oscillations represent the prototype of periodic behaviour associated with enzyme regulation[3,13,14]. Early models for glycolytic oscillations were centred around the reaction catalysed by phosphofructokinase, and took into account the allosteric and regulatory properties of this product-activated enzyme[3,14,81]. More detailed models[82–84] take into account the full set of glycolytic enzyme reactions. In these models, the primary role played by phosphofructokinase in the instability-generating mechanism is somewhat blurred. This example illustrates the two main approaches followed in computational biology. The first is based on minimal models — a complex system is decomposed into simpler modules[85], each of which can be modelled by simple equations. Once these are understood, they are assembled into increasingly complex networks that can exhibit collective properties not apparent in the modules' behaviour. The second relies on large-scale models that aim at incorporating from the outset all known details about the variables and processes of interest. This approach may someday lead to the construction of an electronic cell *in silico*, although that day remains far off. With models as with maps, I believe that an intermediate scale will often prove most fruitful.

The comparison of theoretical predictions with experimental results calls for more quantitative data in molecular and cell biology[86]. The advent of new tools should facilitate the collection of more quantitative data on the dynamics of cellular processes. Such data will complement qualitative studies on the nature of interactions in cellular regulatory networks.

Clarification of the molecular mechanisms underlying oscillations is but one application of computational biology to the study of cellular rhythms. As discussed in this review, models are also used to address the function of these rhythms, which is often related to their frequency encoding, and a variety of related phenomena such as propagating waves and complex oscillations. The link between intracellular oscillations and the propagation of intra- or intercellular waves is well illustrated by Ca^{2+} signalling in many cell types and by cAMP signalling in *Dictyostelium* amoebae. Recent observations on the occurrence of intracellular waves in activated leukocytes[87] provide a challenge for modelling studies. This spatiotemporal phenomenon seems to be linked with the occurrence of metabolic oscillations, the nature of which are unclear. The modelling approach has been applied to account for the transition from simple to complex oscillatory behaviour in the peroxidase reaction[68] and in the Ca^{2+} signalling system[22,66,70,71]. The observation of a transition to chaos in the glycolytic cycle in yeast cell cultures[88] remains to be studied in a similar manner.

Models for cellular rhythms illustrate the roles and advantages of computational biology. First and foremost, modelling takes over when pure intuition reaches its limits. This situation commonly arises when studying cellular processes that involve a large number of variables coupled through multiple regulatory interactions. Here one cannot make reliable predictions on the basis of verbal reasoning. But mathematical models can show the precise parameter ranges that give rise to sustained oscillations. Models also help clarify the molecular mechanisms of these oscillations. Indeed, simulations allow rapid determination of the qualitative and quantitative effects of each parameter, and thereby can help to identify key parameters that have the most profound effect on the system's dynamics. Testing various models permits swift exploration of different mechanisms over a large range of conditions. One of the main roles of models will be to provide a unified conceptual framework to account for experimental observations and to generate testable predictions.

From a more global perspective, which represents one of the strengths of the theoretical approach, the common mathematical structure of models underlines the links between similar dynamic phenomena occurring in widely different biological settings, from genetic to metabolic and neural networks, and from cell to animal populations.   □

1. Fessard, A. *Propriétés Rythmiques de la Matière Vivante* (Hermann, Paris, 1936).
2. Winfree, A. T. *The Geometry of Biological Time* 2nd edn (Springer, New York, 2001).
3. Goldbeter, A. *Biochemical Oscillations and Cellular Rhythms. The Molecular Bases of Periodic and Chaotic Behaviour* (Cambridge Univ. Press, Cambridge, 1996).
4. Volterra, V. Fluctuations in the abundance of a species considered mathematically. *Nature* **118**, 558–560 (1926).

5. Hodgkin, A. L. & Huxley, A. F. A quantitative description of membrane currents and its application to conduction and excitation in nerve. *J. Physiol. (Lond.)* **117,** 500–544 (1952).

6. Koch, C. & Segev, I. (eds) *Methods in Neuronal Modeling. From Synapses to Networks* 2nd edn (MIT Press, Cambridge, MA, 1998).

7. Keener, J. P. & Sneyd, J. *Mathematical Physiology* (Springer, New York, 1998).

8. Noble, D. Modeling the heart—from genes to cells to the whole organ. *Science* **295,** 1678–1682 (2002).

9. Nicolis, G. & Prigogine, I. *Self-Organization in Nonequilibrium Systems. From Dissipative Structures to Order through Fluctuations* (Wiley, New York, 1977).

10. Thomas, R. & d'Ari, R. *Biological Feedback* (CRC Press, Boca Raton, FL, 1990).

11. Ferrell, J. E. Jr Self-perpetuating states in signal transduction: positive feedback, double-negative feedback and bistability. *Curr. Opin. Cell Biol.* **14,** 140–148 (2002).

12. Doedel, E. J. AUTO: A program for the automatic bifurcation analysis of autonomous systems. *Cong. Numer.* **30,** 265–284 (1981). (Available at <http://ftp.cs.concordia.ca/pub/doedel/auto/>.)

13. Hess, B. & Boiteux, A. Oscillatory phenomena in biochemistry. *Annu. Rev. Biochem.* **40,** 237–258 (1971).

14. Goldbeter, A. & Caplan, S. R. Oscillatory enzymes. *Annu. Rev. Biophys. Bioeng.* **5,** 449–476 (1976).

15. Berridge, M. J. Elementary and global aspects of calcium signalling. *J. Physiol. (Lond.)* **499,** 291–306 (1997).

16. Meyer, T. & Stryer, L. Molecular model for receptor-stimulated calcium spiking. *Proc. Natl Acad. Sci. USA* **85,** 5051–5055 (1988).

17. Goldbeter, A., Dupont, G. & Berridge, M. J. Minimal model for signal-induced Ca^{2+} oscillations and for their frequency encoding through protein phosphorylation. *Proc. Natl Acad. Sci. USA* **87,** 1461–1465 (1990).

18. De Young, G. W. & Keizer, J. A single-pool inositol 1,4,5-trisphosphate-receptor-based model for agonist-stimulated oscillations in Ca^{2+} concentration. *Proc. Natl Acad. Sci. USA* **89,** 9895–9899 (1992).

19. Dupont, G. & Goldbeter, A. Properties of intracellular Ca^{2+} waves generated by a model based on Ca^{2+}-induced Ca^{2+} release. *Biophys. J.* **67,** 2191–2204 (1994).

20. Sneyd, J., Charles, A. C. & Sanderson, M. J. A model for the propagation of intercellular calcium waves. *Am. J. Physiol.* **266,** C293–C302 (1994).

21. Dupont, G. *et al.* Mechanism of receptor-oriented intercellular calcium wave propagation in hepatocytes. *FASEB J.* **14,** 279–289 (2000).

22. Schuster, S., Marhl, M. & Höfer, T. Modelling of simple and complex calcium oscillations. From single-cell responses to intercellular signalling. *Eur. J. Biochem.* **269,** 1333–1355 (2002).

23. Swillens, S., Dupont, G., Combettes, L. & Champeil, P. From calcium blips to calcium puffs: theoretical analysis of the requirements for interchannel communication. *Proc. Natl Acad. Sci. USA* **96,** 13750–13755 (1999).

24. Spitzer, N. C., Lautermilch, N. J., Smith, R. D. & Gomez, T. M. Coding of neuronal differentiation by calcium transients. *BioEssays* **22,** 811–817 (2000).

25. De Koninck, P. & Schulman, H. Sensitivity of CaM kinase II to the frequency of Ca^{2+} oscillations. *Science* **279,** 227–230 (1998).

26. Gorbunova, Y. V. & Spitzer, N. C. Dynamic interactions of cyclic AMP transients and spontaneous Ca^{2+} spikes. *Nature* **418,** 93–96 (2002).

27. Dormann, D., Kim, J. Y., Devreotes, P. N. & Weijer, C. J. cAMP receptor affinity controls wave dynamics, geometry and morphogenesis in *Dictyostelium. J. Cell Sci.* **114,** 2513–2523 (2001).

28. Martiel, J. L. & Goldbeter, A. A model based on receptor desensitization for cyclic AMP signaling in *Dictyostelium* cells. *Biophys. J.* **52,** 807–828 (1987).

29. Tang, Y. & Othmer, H. G. Excitation, oscillations and wave propagation in a G-protein-based model of signal transduction in *Dictyostelium discoideum. Phil. Trans. R. Soc. Lond. B* **349,** 179–195 (1995).

30. Palsson, E. & Cox, E. C. Origin and evolution of circular waves and spirals in *Dictyostelium discoideum* territories. *Proc. Natl Acad. Sci. USA* **93,** 1151–1155 (1996).

31. Lauzeral, J., Halloy, J. & Goldbeter, A. Desynchronization of cells on the developmental path triggers the formation of spiral waves of cAMP during *Dictyostelium* aggregation. *Proc. Natl Acad. Sci. USA* **94,** 9153–9158 (1997).

32. Bretschneider, T., Siegert, F. & Weijer, C. J. Three-dimensional scroll waves of cAMP could direct cell movement and gene expression in *Dictyostelium* slugs. *Proc. Natl Acad. Sci. USA* **92,** 4387–4391 (1995).

33. Chadwick, D. J. & Goode, J. A. (eds) *Mechanisms and Biological Significance of Pulsatile Hormone Secretion (Novartis Found. Symp. 227)* (Wiley, Chichester, 2000).

34. Knobil, E. Patterns of hormone signals and hormone action. *New Engl. J. Med.* **305,** 1582–1583 (1981).

35. Belchetz, P. E., Plant, T. M., Nakai, Y., Keogh, E. J. & Knobil, E. Hypophysial responses to continuous and intermittent delivery of hypothalamic gonadotropin-releasing hormone. *Science* **202,** 631–633 (1978).

36. Hindmarsh, P. C., Stanhope, R., Preece, M. A. & Brook, C. G. D. Frequency of administration of growth hormone—an important factor in determining growth response to exogenous growth hormone. *Horm. Res.* **33** (Suppl. 4), 83–89 (1990).

37. Tornheim, K. Are metabolic oscillations responsible for normal oscillatory insulin secretion? *Diabetes* **46,** 1375–1380 (1997).

38. Li, Y. X. & Goldbeter, A. Frequency specificity in intercellular communication: the influence of patterns of periodic signalling on target cell responsiveness. *Biophys. J.* **55,** 125–145 (1989).

39. Goldbeter, A., Dupont, G. & Halloy, J. The frequency encoding of pulsatility. *Novartis Found. Symp.* **227,** 19–36 (2000).

40. Wagner, C., Caplan, S. R. & Tannenbaum, G. S. Genesis of the ultradian rhythm of GH secretion: a new model unifying experimental observations in rats. *Am. J. Physiol.* **275,** E1046–E1054 (1998).

41. Maki, L. W. & Keizer, J. Mathematical analysis of a proposed mechanism for oscillatory insulin secretion in perifused HIT-15 cells. *Bull. Math. Biol.* **57,** 569–591 (1995).

42. Dunlap, J. C. Molecular bases for circadian clocks. *Cell* **96,** 271–290 (1999).

43. Young, M. W. & Kay, S. A. Time zones: a comparative genetics of circadian clocks. *Nature Rev. Genet.* **2,** 702–715 (2001).

44. Reppert, S. M. & Weaver, D. R. Coordination of circadian timing in mammals. *Nature* **418,** 935–941 (2002).

45. Hardin, P. E., Hall, J. C. & Rosbash, M. Feedback of the *Drosophila* period gene product on circadian cycling of its messenger RNA levels. *Nature* **343,** 536–540 (1990).

46. Kronauer, R. E., Forger, D. B. & Jewett, M. E. Quantifying human circadian pacemaker response to brief, extended, and repeated light stimuli over the phototopic range. *J. Biol. Rhythms* **14,** 500–515 (1999).

47. Gonze, D., Roussel, M. & Goldbeter, A. A model for the enhancement of fitness in cyanobacteria based on resonance of a circadian oscillator with the external light-dark cycle. *J. Theor. Biol.* **214,** 577–597 (2002).

48. Goodwin, B. C. Oscillatory behavior in enzymatic control processes. *Adv. Enzyme Regul.* **3,** 425–438 (1965).

49. Ruoff, P., Vinsjevik, M., Monnerjahn, C. & Rensing, L. The Goodwin model: simulating the effect of light pulses on the circadian sporulation rhythm of *Neurospora crassa. J. Theor. Biol.* **209,** 29–42 (2001).

50. Goldbeter, A. A model for circadian oscillations in the *Drosophila period* protein (PER). *Proc. R. Soc. Lond. B* **261,** 319–324 (1995).

51. Leloup, J. C. & Goldbeter, A. A model for circadian rhythms in *Drosophila* incorporating the formation of a complex between the PER and TIM proteins. *J. Biol. Rhythms* **13,** 70–87 (1998).

52. Tyson, J. J., Hong, C. I., Thron, C. D. & Novak, B. A simple model of circadian rhythms based on dimerization and proteolysis of PER and TIM. *Biophys. J.* **77,** 2411–2417 (1999).

53. Glossop, N. R., Lyons, L. C. & Hardin, P. E. Interlocked feedback loops within the *Drosophila* circadian oscillator. *Science* **286,** 766–768 (1999).

54. Shearman, L. P. *et al.* Interacting molecular loops in the mammalian circadian clock. *Science* **288,** 1013–1019 (2000).

55. Preitner, N. *et al.* The orphan nuclear receptor REV-ERBα controls circadian transcription within the positive limb of the mammalian circadian oscillator. *Cell* **110,** 251–260 (2002).

56. Ueda, H. R., Hagiwara, M. & Kitano, H. Robust oscillations within the interlocked feedback model of *Drosophila* circadian rhythm. *J. Theor. Biol.* **210,** 401–406 (2001).

57. Smolen, P., Baxter, D. A. & Byrne, J. H. Modeling circadian oscillations with interlocking positive and negative feedback loops. *J. Neurosci.* **21,** 6644–6656 (2001).

58. Leloup, J. C. & Goldbeter, A. Towards a detailed computational model for the mammalian circadian clock. *Proc. Natl Acad. Sci. USA* (submitted).

59. Richardson, G. S. & Malin, H. V. Circadian rhythm sleep disorders: pathophysiology and treatment. *J. Clin. Neurophysiol.* **13,** 17–31 (1996).

60. Toh, K. L. *et al.* An hPer2 phosphorylation site mutation in familial advanced sleep phase syndrome. *Science* **291,** 1040–1043 (2001).

61. Ebisawa, T. *et al.* Association of structural polymorphisms in the human *period3* gene with delayed sleep phase syndrome. *EMBO Rep.* **2,** 342–346 (2001).

62. Leloup, J. C. & Goldbeter, A. A molecular explanation for the long-term suppression of circadian rhythms by a single light pulse. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **280,** R1206–R1212 (2001).

63. Barkai, N. & Leibler, S. Circadian clocks limited by noise. *Nature* **403,** 267–268 (2000).

64. Gonze, D., Halloy, J. & Goldbeter, A. Robustness of circadian rhythms with respect to molecular noise. *Proc. Natl Acad. Sci. USA* **99,** 673–678 (2002).

65. Vilar, J. M., Kueh, H. Y., Barkai, N. & Leibler, S. Mechanisms of noise-resistance in genetic oscillators. *Proc. Natl Acad. Sci. USA* **99,** 5988–5992 (2002).

66. Goldbeter, A. *et al.* From simple to complex oscillatory behavior in metabolic and genetic control networks. *Chaos* **11,** 247–260 (2001).

67. Rinzel, J. A formal classification of bursting mechanisms in excitable systems. *Lect. Notes Biomath.* **71,** 267–281 (1987).

68. Olsen, L. F. & Degn, H. Chaos in biological systems. *Q. Rev. Biophys.* **18,** 165–225 (1985).

69. Glass, L. & Mackey, M.C. *From Clocks to Chaos: The Rhythms of Life* (Princeton Univ. Press, Princeton, 1988).

70. Shen, P. & Larter, R. Chaos in intracellular Ca^{2+} oscillations in a new model for non-excitable cells. *Cell Calcium* **17,** 225–232 (1995).

71. Kummer, U. *et al.* Switching from simple to complex oscillations in calcium signaling. *Biophys. J.* **79,** 1188–1195 (2000).

72. Goldbeter, A. A minimal cascade model for the mitotic oscillator involving cyclin and cdc2 kinase. *Proc. Natl Acad. Sci. USA* **88,** 9107–9111 (1991).

73. Novak, B. & Tyson, J. J. Numerical analysis of a comprehensive model of M-phase control in *Xenopus* oocyte extracts and intact embryos. *J. Cell Sci.* **106,** 1153–1168 (1993).

74. Tyson, J. J. & Novak, B. Regulation of the eukaryotic cell cycle: molecular antagonism, hysteresis, and irreversible transitions. *J. Theor. Biol.* **210,** 249–263.

75. Tyson, J. J., Chen, K. & Novak, B. Network dynamics and cell physiology. *Nature Rev. Mol. Cell Biol.* **2,** 908–916 (2001).

76. Elowitz, M. B. & Leibler, S. A synthetic oscillatory network of transcriptional regulators. *Nature* **403,** 335–338 (2000).

77. Gardner, T. S., Cantor, C. R. & Collins, J. J. Construction of a genetic toggle switch in *Escherichia coli. Nature* **403,** 339–342 (2000).

78. Lev Bar-Or, R. *et al.* Generation of oscillations by the p53-Mdm2 feedback loop: a theoretical and experimental study. *Proc. Natl Acad. Sci. USA* **97,** 11250–11255 (2000).

79. Maroto, M. & Pourquié, O. A molecular clock involved in somite segmentation. *Curr. Top. Dev. Biol.* **51,** 221–248 (2001).

80. Jacquet, M., Renault, G., Lallet, S., de Mey, J. & Goldbeter, A. Oscillatory nucleocytoplasmic shuttling of the general stress response transcriptional activator Msn2 in *Saccharomyces cerevisiae. Nature* (submitted).

81. Boiteux, A., Goldbeter, A. & Hess, B. Control of oscillating glycolysis of yeast by stochastic, periodic, and steady source of substrate: a model and experimental study. *Proc. Natl Acad. Sci. USA* **72,** 3829–3833 (1975).

82. Termonia, Y. & Ross, J. Oscillations and control features in glycolysis: numerical analysis of a comprehensive model. *Proc. Natl Acad. Sci. USA* **78,** 2952–2956 (1981).

83. Hynne, F., Dano, S. & Sorensen, P. G. Full-scale model of glycolysis in *Saccharomyces cerevisiae. Biophys. Chem.* **94,** 121–163 (2001).

84. Reijenga, K. A., Westerhoff, H. V., Kholodenko, B. N. & Snoep, J. L. Control analysis for autonomously oscillating biochemical networks. *Biophys. J.* **82,** 99–108 (2002).

85. Hartwell, L. H., Hopfield, J. J., Leibler, S. & Murray, A. W. From molecular to modular cell biology. *Nature* **402** (Suppl.), C47–C52 (1999).

86. Koshland, D. E. Jr The era of pathway quantification. *Science* **280,** 852–853 (1998).

87. Petty, H. R. Neutrophil oscillations: temporal and spatiotemporal aspects of cell behavior. *Immunol. Res.* **23,** 85–94 (2001).

88. Nielsen, K., Sörensen, P. G. & Hynne, F. Chaos in glycolysis. *J. Theor. Biol.* **186,** 303–306 (1997).

89. Honma, S. *et al. Dec1* and *Dec2* are regulators of the mammalian molecular clock. *Nature* **419,** 841–844 (2002).

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