# Modelling biochemical oscillations and cellular rhythms

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**The usefulness of theoretical models for the study of biological rhythms is illustrated by considering several examples of oscillations resulting from different modes of cellular regulation. Considered in turn are oscillations resulting from the regulation of enzyme activity, receptor function, transport, and gene expression. The generation of rhythmic behaviour by these various types of regulation is exemplified, respectively, by glycolytic oscillations in yeast cells, the periodic synthesis of cyclic AMP in *Dictyostelium* amoebae, intracellular calcium oscillations, and circadian rhythms in *Drosophila*.**

OSCILLATORY phenomena have been observed and studied theoretically in chemical systems[1,2], but appear to be particularly widespread in biological systems[3]. The usefulness of mathematical models in theoretical biology is well illustrated by the study of oscillatory phenomena in biological systems. The purpose of this paper is to show how different modes of cellular regulation are capable of giving rise to sustained oscillatory behaviour. After a brief presentation of biological rhythms and their molecular mechanisms, considered in turn will be examples of oscillations resulting from the regulation of enzymatic activity, receptor function, transport, and gene expression. Theoretical models based on experimental observations will thus be described for glycolytic oscillations in yeast cells, the periodic synthesis of cyclic AMP in *Dictyostelium* amoebae, intracellular calcium oscillations, and circadian rhythms in *Drosophila*. The theoretical approach throws light on the properties shared by these various rhythms characterized by widely different periods.

## The molecular mechanism of biological rhythms

A nonexhaustive list of the main biological rhythms is given in Table 1, where the various rhythms are classified according to their increasing period[3]. The sign * means that the rhythm can be observed at the level of an isolated cell; this does not exclude the possibility that the rhythm may originate from interactions between different cells, as occurs, for example, in a network of neurons coupled by activating or inhibitory interactions.

The most rapid rhythms are those observed in neurons, with periods ranging from $10^{-2}$ to 10 s (ref. 4). The generation of trains of periodic action potentials is well understood: the phenomenon results from the interactions between several voltage-dependent ionic conductances. Neurons, as well as muscle (among which cardiac) cells, are electrically excitable: an action potential is generated when a suprathreshold depolarizing stimulus causes the membrane potential to abruptly change its polarity before returning to its stable, resting value. In certain conditions, e.g. in the presence of a constant depolarizing current, such an excitability transforms into the generation of repetitive action potentials at regular intervals. These oscillations of the membrane potential appear to play a key role in the functioning of the brain, e.g. in the processing of sensory information. From a theoretical point of view, neural rhythms can be described in terms of equations of the Hodgkin–Huxley type, first used to account for the electrical excitability of the squid giant axon in terms of the sodium and potassium conductances.

The cardiac rhythm originates from the autonomous

**Table 1.** Main biological rhythms. The sign * indicates that the rhythm can (already) be observed at the cellular level

| | Period |
|---|---|
| Neural rhythms* | 0.01 to 10 s (and more?) |
| Cardiac rhythm* | 1 s |
| Calcium oscillations* | 1 s to min |
| Biochemical oscillations* | 1 min to 20 min |
| Mitotic cycle* | 10 min to 24 h or more |
| Hormonal rhythms* | 10 min to 5 h (also 24 h) |
| Circadian rhythms* | 24 h |
| Ovarian cycle | 28 days (human) |
| Annual rhythms | 1 year |
| Epidemics and ecological oscillations | years |

periodic electrical activity of specialized tissues of the heart, such as the sinus or the auriculo-ventricular nodes. At the cellular level, the mechanisms of oscillatory behaviour are largely similar to those underlying neuronal oscillations.

Besides these oscillations of electrical origin, other rhythms originate from the various modes of cellular regulation. Thus, several examples of oscillatory enzyme reactions are known, with a period of the order of a few minutes[5,6]. The mitotic oscillator which controls the eukaryotic cell division cycle is an important example of biochemical oscillator involving a cascade of enzymatic reactions regulated by phosphorylation–dephosphorylation, which culminates in the periodic activation of the protein kinase cdc2. The period of the mitotic oscillator varies from several minutes in some embryonic cells up to 24 h or more in somatic cells[7]. Theoretical models for the mitotic control system have been proposed (see ref. 3 for review).

Oscillations of intracellular calcium of a period ranging from seconds to minutes have been observed since 1985 in a large variety of cell types[8]. Besides oscillations of this intracellular messenger, oscillations of intercellular messengers are known, such as the periodic synthesis of cyclic AMP in *Dictyostelium* amoebae[9], or the pulsatile secretion of a large number of hormones, with periods ranging from some 10 min for insulin, up to 3 h for the growth hormone.

Circadian rhythms, which have a period of about 24 h, are observed in nearly all living organisms, including some bacterial species. Significant experimental advances have been made in recent years as to the molecular mechanisms of these rhythms, particularly in organisms such as *Drosophila*[10].

Other rhythms possess a supracellular mechanism involving regulatory interactions between different organs, as is the case for the ovarian cycle which has a period of about 28 days in the human female, or between different animal species. Predator–prey oscillations represent the first periodic phenomenon that has been studied in a theoretical manner by means of mathematical models, in the first quarter of this century. The study of these oscillations remains a classical problem in theoretical ecology. A particular case is that of epidemics which recur periodically, owing to the interactions between an infectious agent and a population of susceptible hosts which develop an immune response of variable duration against the pathological agent.

Returning to biochemical rhythms of a nonelectrical nature, we will examine by means of a few selected examples how oscillations originate from the different modes of cellular regulation. We shall also mention the conditions in which simple periodic behaviour gives rise to more complex oscillatory phenomena, including chaos.

## Cellular regulations and biological rhythms

The appropriate spatio-temporal coordination of thousands of biochemical reactions requires the operation of multiple modes of cellular regulation exerted on the activity of enzymes, the functioning of receptors and transport processes, and gene expression associated with the synthesis of particular proteins. Each of these types of cellular regulation can give rise to rhythmic behaviour.

### *Enzymatic regulation: Glycolytic oscillations*

Glycolysis is an important metabolic pathway, the function of which is to synthesize ATP upon degradation of a sugar such as glucose. It is known for four decades that upon addition of glucose, damped oscillations of NADH (a glycolytic intermediate whose fluorescence can be recorded in a continuous manner) occur in a yeast cell suspension. Experiments carried out in yeast extracts later showed that these oscillations become sustained when a glycolytic substrate such as glucose or fructose is injected at a constant rate. Glycolytic oscillations remain the prototype of periodic behaviour due to the regulation of enzyme activity[3,5,6].

The period of the phenomenon decreases from about 8 to 3 min when the substrate input rate increases. Moreover, sustained oscillations occur in a window bounded by two critical values of this control parameter: below 20 mM/h and above 160 mM/h, the system evolves towards a stable steady state.

Glycolysis consists of a chain of enzymatic reactions leading from hexokinase to phosphofructokinase (PFK), then to other reactions eventually producing, in yeast, ethanol and $CO_2$. Very early on, experiments showed that PFK plays a primary role in the generation of glycolytic oscillations (see refs 3, 5, 6 for review). The latter indeed disappear as soon as an intermediate following the step catalysed by PFK (e.g. fructose 1,6-bisphosphate) is used as glycolytic substrate.

What are the particular properties of PFK which enable this enzyme to generate (in conjunction with a source and a sink) metabolic oscillations? While negative feedback processes are by far the most common in enzymatic regulation, PFK is subject to positive feedback regulation by a reaction product, ADP, and by AMP produced from ADP. The PFK reaction is therefore autocatalytic since the rate of the reaction increases as the product concentration rises. In addition to the nonlinearity associated with this positive feedback process, the PFK kinetics possesses a sigmoidal character due to the allosteric nature of the enzyme: PFK contains several subunits which exist in two conformational states differing by the affinity for the substrate and/or the catalytic

activity. A phenomenon of cooperativity characterizes the transition from the less active $T$ to the more active $R$ conformation; this transition is either concerted or sequential. Binding of the product to a regulatory site induces the transition from the $T$ to the $R$ state of the enzyme.

The analysis of a mathematical model for a reaction catalysed by an allosteric enzyme activated by its reaction product (schematized in Figure 1 *a*) allows one to better comprehend the mechanism of glycolytic oscillations generated by PFK[3,6]. The model is described by a system of two ordinary differential equations, the nonlinearity of which results from the positive feedback exerted by the reaction product and from the cooperativity of the enzyme. The two variables considered are the substrate and product concentrations. The main control parameters are the substrate injection rate, $v$, and parameters linked to the enzyme, such as its concentration, its maximum rate, and the allosteric constant that measures the ratio of enzyme in the $T$ and $R$ states in the absence of ligand.

The study of the dynamic behaviour of the model shows that below a critical value of $v$ and above a second, higher critical value of this parameter, the system evolves towards a stable steady state. In agreement with experimental observations, sustained oscillations of the substrate and product concentrations occur in the window bounded by the two critical values of $v$. In the phase plane where the concentration of substrate is plotted versus that of the product, these oscillations correspond to the evolution towards a closed curve, the limit cycle, surrounding the unstable steady state. Here, the limit cycle is unique and can be reached regardless of initial conditions. This ensures the stability of this type of periodic behaviour in regard to fluctuations.

The model yields the following description of the oscillatory mechanism in terms of molecular events: starting from a low level of product, the enzyme is predominantly in the less active state, $T$; the substrate accumulates at a constant rate $v$ and is slowly transformed

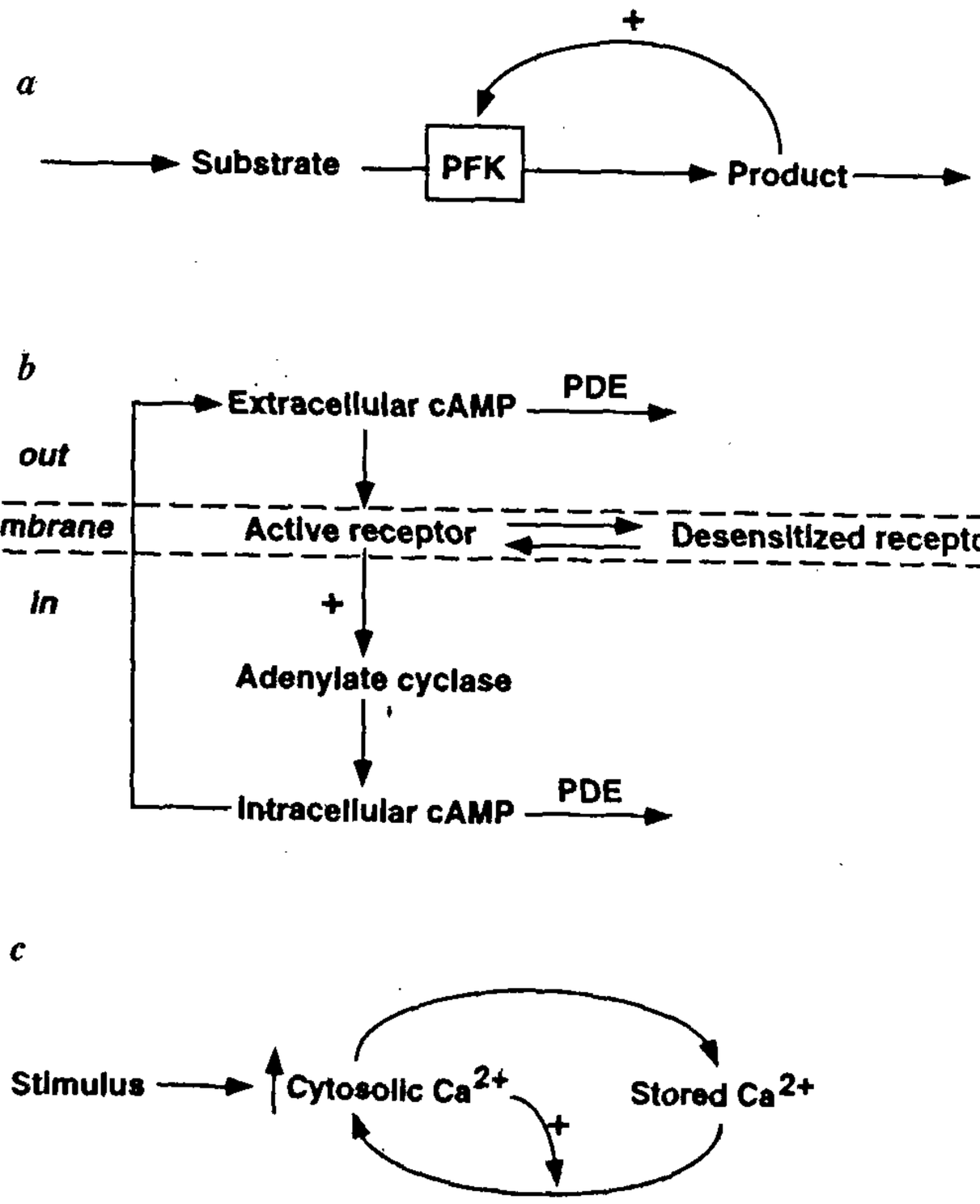


**Figure 1.** Regulatory mechanisms generating oscillatory behaviour. ***a***, The activation of the allosteric enzyme phosphofructokinase (PFK) by a reaction product gives rise to glycolytic oscillations. ***b***, The mechanism of periodic synthesis of cAMP in the slime mould *Dictyostelium discoideum* involves the activation of adenylate cyclase following the binding of extracellular cAMP to its membrane receptor as well as receptor desensitization, and cAMP hydrolysis by phosphodiesterase (PDE). ***c***, Calcium-induced calcium release (CICR) is at the heart of the mechanism of intracellular calcium oscillations triggered in a variety of cells by an external stimulus.

by the small amount of enzyme in the *R* conformation. The product concentration progressively rises until it reaches the threshold level beyond which it triggers the cooperative transition of the enzyme subunits from the *T* to the more active *R* conformation. The positive feedback loop gives rise to an abrupt peak in the synthesis of product; the latter eventually begins to decrease, however, owing to two concomitant processes. First, the product is itself consumed in a sink reaction without which oscillations would not occur. Second, the substrate is consumed upon its transformation into reaction product; the drop in substrate is followed by a drop in product synthesis. The enzyme then returns to its less active *T* state. A new cycle of the oscillations begins when the substrate resumes its accumulation, as soon as the substrate input $v$ exceeds the rate of substrate transformation by the enzyme in its less active conformation.

Whereas simple periodic behaviour of the limit cycle type is the only one that can be observed in this model, more complex oscillatory phenomena occur as soon as two instability-generating mechanisms interact within the same system[3]. Thus, when two autocatalytic enzymatic reactions are coupled in series, the variety of modes of dynamic behaviour in such a three-variable system is greatly increased[11]. Depending on the values of a single control parameter, we can observe simple periodic oscillations, the coexistence between two regimes of stable periodic oscillations of the limit cycle type, and aperiodic oscillations in the form of chaos. The latter oscillations correspond to the evolution towards a strange attractor in the phase space: the oscillatory system remains confined in a given region of the phase space without ever passing again through any point of the trajectory, as would occur in the case of a limit cycle.

The coexistence between two stable limit cycles illustrates well the interest of a theoretical approach to biological rhythms. This phenomenon, referred to as *birhythmicity*[11], was predicted theoretically before being observed experimentally in a system of coupled oscillatory chemical reactions[12]. Birhythmicity has not yet been observed experimentally in biological systems, in contrast to the coexistence between two stable steady states, known as *bistability*, for which numerous experimental examples are known in chemistry and biology.

While three variables at least are needed to obtain chaos, birhythmicity can already be observed in a two-variable system, as in the two-variable model of an autocatalytic enzyme reaction proposed for glycolytic oscillations, when this model is extended to take into account a nonlinear recycling of product into substrate[3].

### *Receptor regulation: Oscillations of cyclic AMP in Dictyostelium amoebae*

The generation of periodic signals of cyclic AMP (cAMP) in the amoebae *Dictyostelium discoideum* represents a model of choice for pulsatile intercellular communication in higher organisms. Following starvation, these amoebae collect around aggregation centers by a chemotactic response to cAMP signals emitted by the centers with a periodicity of about 5 min. On agar, the amoebae aggregate around the centers by forming concentric or spiral waves. Experiments in stirred cell suspensions confirm the periodic nature of cAMP synthesis in this slime mould[9].

The mechanism responsible for the pulsatile generation of cAMP signals again involves a positive feedback loop: extracellular cAMP binds to a cell surface receptor and thereby activates the enzyme adenylate cyclase which catalyses the synthesis of cAMP from ATP. Intracellular cAMP thus produced is secreted into the extracellular medium where it can bind again to the receptor. This mechanism of self-amplification would lead to a biochemical 'explosion' were it not for limiting factors that counteract the effect of autocatalysis. In the case of glycolytic oscillations, substrate consumption plays such a limiting role. In *Dictyostelium*, receptor regulation is the process that limits the self-amplification in cAMP synthesis. As soon as cAMP binds to the active form of the receptor, the latter is phosphorylated. This reversible phosphorylation accompanies the transition of the receptor into a desensitized state unable to elicit the activation of adenylate cyclase. Experiments indicate that cAMP oscillations are accompanied by a periodic alternance of the receptor between the active (non-phosphorylated) and desensitized (phosphorylated) states[13].

The analysis of a mathematical model based on self-amplification in cAMP synthesis and on the reversible desensitization of the cAMP receptor (see scheme in Figure 1 *b*) allows one to determine the conditions in which this system of intercellular communication operates in a periodic manner[3,14]. The model, described by a system of three nonlinear kinetic equations, gives rise to sustained oscillations in the concentrations of intracellular and extracellular cAMP, and in the fraction of active, nonphosphorylated receptor.

This model not only accounts for the periodic nature of cAMP synthesis but also provides an explanation for the onset of cAMP oscillations in the course of *Dictyostelium* development. Soon after starvation, amoebae are not capable of amplifying cAMP signals; after a few hours, amoebae begin to relay these signals by amplifying them in a pulsatile manner. Still a few hours later, the amoebae acquire the capability of generating pulsatile cAMP signals in a periodic, autonomous manner. The theoretical model shows that these developmental transitions *no relay–relay–oscillations* correspond to transitions between different modes of dynamic behaviour resulting from the continuous increase in the

control parameters measuring the activity of adenylate cyclase and phosphodiesterase, the enzymes that synthesize and degrade cAMP, respectively. The cells would thus follow a *developmental path* in this parameter space; cells most advanced on such a path would be the first to enter the oscillatory domain and would become aggregation centers capable of releasing autonomously periodic signals of cAMP. This explanation bears, more generally, on the ontogenesis of biological rhythms, as it shows how the continous variation of biochemical parameters or ionic conductances can lead to the passage through a bifurcation point corresponding to the onset of periodic behaviour in the course of development.

The cAMP signalling system in *Dictyostelium* can also serve as model for intercellular communications of a pulsatile nature. Thus, most hormones are secreted not constantly but rather in a pulsatile manner, with a periodicity ranging from about 10 min up to several hours; such oscillations of relatively high frequency are often superimposed on a slower, circadian variation. The prototype of pulsatile hormone secretion is that of GnRH (also referred to as LHRH). This decapeptide hormone, secreted by the hypothalamus with a periodicity of 1 h in the rhesus monkey and in man, induces the secretion by the pituitary of the gonadotropic hormones LH and FSH.

In *Dictyostelium*, the signals of cAMP are encoded in terms of their frequency: while signals emitted every 5 min induce cell aggregation and differentiation, constant signals or cAMP pulses emitted every 2 min fail to have such physiological effects. The model shows that if the receptor has enough time to resensitize when the interval separating two pulses is of 5 min, it cannot resensitize sufficiently when the interval is of 2 min only, or when the signal is applied in a continuous manner[3]. Similarly, the studies carried out by the group of E. Knobil have shown that the frequency of GnRH secretion governs the physiological efficacy of the hormone: while a GnRH signal emitted once an hour induces the normal levels of LH and FSH required for ovulation, signals of a periodicity of 30 min or 2 h, as well as constant GnRH signals, remain ineffective. This observation led Knobil to conclude that the temporal profile of the hormone is as important, if not more, than its concentration in the blood. Such results have led to clinical applications. Thus, certain women suffering from troubles of GnRH secretion are sterile because of impaired ovulation. Implanting these patients with pumps releasing GnRH at the physiological frequency restores the levels of gonadotropic hormones required for inducing ovulation.

### *Transport regulation: Oscillations of intracellular calcium*

Among cellular rhythms discovered in recent years, few are as important and widespread as calcium oscillations[8]. These oscillations were first demonstrated in 1985 in mouse eggs upon fertilization, and were since observed in a large variety of cell types (hepatocytes, cardiac or pancreatic cells, pituitary gonadotrophs, . . .), following stimulation by a hormone or a neurotransmitter. In the fertilized egg, oscillations appear to be triggered by a protein, oscillin, secreted by the spermatozoon upon fusion with the oocyte. In view of their ubiquity and of the role of calcium as intracellular messenger, these oscillations represent one of the most significant advances over the last decade in the field of cellular signalling.

The molecular mechanism of the oscillations relies on the transport of calcium from intracellular stores – the endoplasmic reticulum, or the sarcoplasmic reticulum in muscle and cardiac cells – into the cytosol. When a cell is stimulated by a hormone, the binding of this ligand to its receptor elicits the synthesis of an intracellular messenger, inositol 1,4,5-trisphosphate ($IP_3$), which triggers the release of calcium from the intracellular stores; cytosolic calcium is then pumped back into the stores. Hormonal stimulation thus triggers a transient increase in cytosolic calcium concentration.

The fact that the response to hormonal stimulation often takes the form of sustained calcium oscillations is due to the regulation of calcium transport known as *calcium-induced calcium release* (CICR): cytosolic calcium activates the release of calcium from the intracellular stores (Figure 1 *c*). This regulation also represents an example of receptor regulation, since the $IP_3$ receptor serves as channel allowing the efflux of calcium from the endoplasmic reticulum.

The analysis of theoretical models based on CICR again shows how this nonlinear regulatory process gives rise to an instability of the steady state, associated with the evolution to sustained oscillations, for appropriate values of the control parameters[3,15,16]. The simplest model based on CICR contains two variables, namely the concentrations of cytosolic and intravesicular calcium. This model accounts for the effect of a progressive increase in extracellular stimulation: below a critical value of the external signal (hormone or neurotransmitter), cytosolic calcium reaches a low steady state level. In a window bounded by two critical values of the external stimulus, oscillations occur with a frequency that increases with the level of stimulation. Above the higher critical stimulus value, cytosolic calcium reaches a high steady state level.

### *Genetic regulation: Circadian rhythms in Drosophila*

The origin of circadian rhythms represents a long-standing puzzle in chronobiology. These rhythms, which have a period close to 24 h, are encountered in nearly all

living organisms, including some bacterial species, and possess an important physiological function in allowing the organism to adapt to its periodically changing environment. In humans, many functions vary in a circadian manner, as illustrated for example by the sleep–wake cycle and by nutrition. In view of the circadian variation of a large number of hormones and enzymatic activities, the response of the organism to some drugs may also vary according to the time of the day; this observation sets the biochemical foundations for the rapidly growing field of chronopharmacology.

Like the other oscillatory phenomena mentioned above, circadian rhythms are endogenous, i.e. they originate from regulatory processes within the organism rather than from the periodic variation of the environment. Circadian rhythms indeed persist in constant light or darkness. Among the most conspicuous properties of circadian rhythms are the possibility of entrainment by light–dark or temperature cycles, and the relative independence of their period with respect to temperature, a phenomenon referred to as *temperature compensation*.

In mammals, circadian rhythms are generated by the suprachiasmatic (SCN) nuclei which are groups of neurons located in the hypothalamus. How SCN neurons are able to generate rhythms of 24 h period remains an open question. The phenomenon occurs at the cellular level, since experiments indicate that an isolated SCN neuron retains the circadian variation in electrical activity.

The unraveling of the molecular bases of circadian rhythms has undergone rapid advances, thanks to genetic studies, in organisms such as the fly *Drosophila* or the fungus *Neurospora*[10,17]. A circadian rhythm of locomotor activity has been demonstrated in *Drosophila*. Mutagenesis studies have permitted to isolate 'short' and 'long' period mutants for which the periodicity of the locomotor rhythm has shifted from close to 24 h in the wild type to 19 h and 28 h, respectively[18]. The mutated gene is known as *per* (for 'period') and codes for a protein PER of about 1220 amino acid residues. The 'short' and 'long' period phenotypes both correspond to point mutations in the gene.

The *per* mRNA also varies in a circadian manner, and its peak precedes by several hours the peak in PER. This observation suggested that the mechanism of oscillations involves a negative feedback exerted by PER on the expression of the *per* gene[19]. Such a mechanism for oscillations in the synthesis of a protein and its mRNA was proposed more than 30 years ago by Goodwin[20], shortly after Jacob and Monod laid the molecular foundations of genetic regulation.

Recent studies have shown that the PER protein acts as a regulator of transcription and can thereby influence the expression of a large number of genes. Also involved in the generation of circadian rhythmicity in *Drosophila* is the multiple phosphorylation of PER, which could

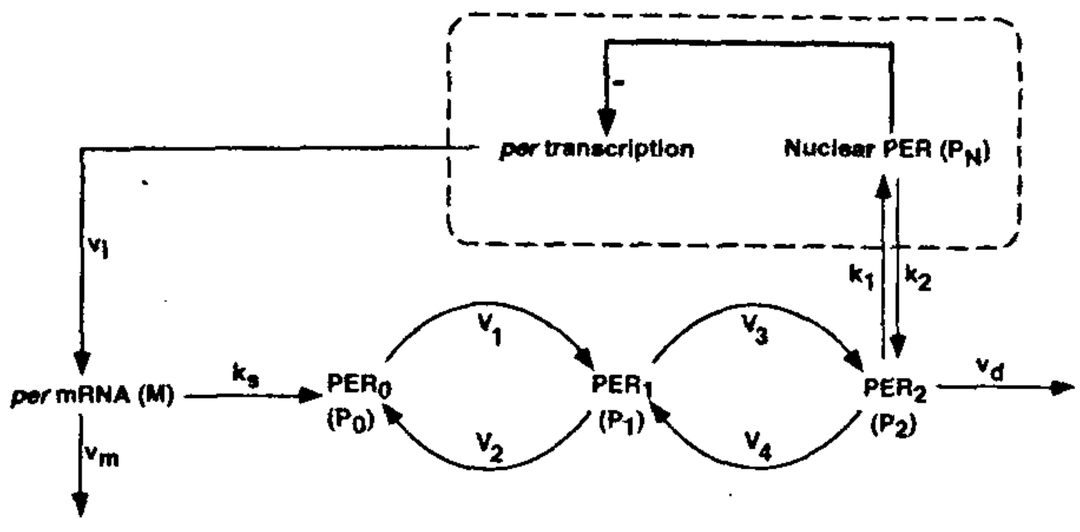


**Figure 2.** Model for circadian oscillations of the PER protein in *Drosophila*[3,21]. The model incorporates the transcription of the *per* gene into *per* mRNA, synthesis of PER followed by two successive phosphorylations of the protein, degradation of PER and its mRNA, PER transport into the nucleus and repression of *per* expression by the nuclear form of PER. An extended version of this model[22] incorporates the role of the TIM protein which forms a complex with PER.

control the degradation of the protein and/or its entry into the nucleus.

A theoretical model based on the control of PER degradation and entry into the nucleus by two successive phosphorylations and on the negative feedback exerted by PER on the expression of the *per* gene (see Figure 2) is described by a system of five nonlinear kinetic equations[3,21]. The numerical integration of these equations shows that sustained oscillations in the concentrations of PER and its mRNA can occur for appropriate parameter values. In agreement with experimental observations, the maximum in *per* mRNA precedes the peak in PER by a few hours. The model shows that the successive phosphorylations of the protein introduce a delay in the negative feedback loop, which favours the occurrence of sustained oscillations.

Besides PER, a second protein, referred to as TIM, encoded by the gene *tim* (for 'timeless'), plays an important role in the mechanism of circadian oscillations in *Drosophila*. The PER and TIM proteins form a complex which migrates from the cytosol into the nucleus where it represses the expression of the *per* and *tim* genes. A theoretical model extended to take into account the formation of the PER–TIM complex as well as the multiple phosphorylation of the two proteins is described by a system of ten kinetic equations[22]. This model yields results similar to those obtained in the simpler model based on regulation by PER alone. However, in addition to simple periodic oscillations, the extended model allows for the occurrence of more complex phenomena such as birhythmicity and chaos. The possible physiological significance of these complex oscillatory phenomena remains questionable, however, since they occur in restricted ranges of parameter values.

The genetic regulatory mechanism involved in the generation of circadian rhythms in *Drosophila* could apply, more generally, to other organisms[10]. Thus, in

*Neurospora*, a similar negative autoregulatory loop has been characterized[23] for the expression of the *frq* ('frequency') gene. It is likely that the regulation of gene expression and protein synthesis suppressed form a central part of the mechanism of circadian rhythms for most organisms. Inhibitors of protein synthesis and of DNA transcription indeed induce phase shifts of circadian rhythms, or even their suppression when the addition of such inhibitors exceeds a threshold level.

## Conclusions

Biochemical as well as neuronal oscillations all result from the instability of a nonequilibrium steady state, beyond which the system evolves towards a limit cycle in the phase space. Processes of positive or negative feedback lie at the heart of the instability-generating mechanism. The interaction between multiple instability-generating mechanisms, often associated with a multiplicity of feedback processes, can lead to more complex oscillatory phenomena including 'bursting' oscillations, the coexistence between two (or more) simultaneously stable periodic regimes (birhythmicity), or aperiodic oscillations (chaos).

The study of theoretical models represents a highly useful approach that complements well the experimental approach on which it is based. The interest of the models is to shed light on the core mechanism capable of generating oscillations – by pinpointing the essential variables as well as their interactions – and on the precise conditions in which oscillations occur. Often indeed, verbal descriptions based on sheer intuition alone do not suffice for predicting the dynamic behaviour of a complex system containing a large number of variables and governed by multiple, nonlinear regulatory interactions.

Finally, the theoretical approach underlines the deep unity of biological rhythms. The latter may originate, a.o., from the regulation of enzyme activity, receptor function, transport processes, or gene expression: regardless of the type of control and the nature of the molecules involved, common properties emerge for the rhythms generated with widely different periods. Positive or/and negative feedback are found to give rise to rhythmic behaviour at all levels of biological organization.


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