Guest Editorial

Biological rhythms: Clocks for all times

Albert Goldbeter

The development and harmonious functioning of an organism depend on the exquisite coordination of myriad intertwined biological processes. As illustrated by this issue of *Current Biology* devoted to the 'biology of time', temporal organization plays crucial roles in coordinating dynamic phenomena as diverse as progression through the cell cycle, the processing of information, adaptation to the periodic environment, and the response to extracellular or intracellular signals. Here I will focus on one of the most conspicuous manifestations of temporal organization, that which takes the form of rhythmic behavior.

If rhythms did not exist, would we see the passage of time? The alternation of day and night, and the cycle of the seasons remind us that life's environment is inherently periodic. Life itself is rhythmic: from the periodic generation of action potentials in neurons or cardiac cells to the cell division cycle and circadian rhythms, many key cellular processes possess a repetitive, oscillatory nature. Rhythmic behavior also occurs at the supracellular level, as exemplified by the ovarian cycle and by annual rhythms such as flowering, migration, hibernation or reproduction in some mammalian species. The period of biological rhythms spans more than ten orders of magnitude, from a fraction of a second up to tens of years (see Table 1).

Why are there so many biological rhythms? The answer is: regulation. Cellular regulatory processes are multifarious and can all give rise to oscillations [1]. Thus, activation and inhibition of ionic conductances as a function of the membrane potential combine to produce periodic behavior in electrically excitable cells. The regulation of enzyme activity underlies metabolic oscillations, while control of transport between different intracellular compartments gives rise to oscillations of cytosolic Ca^{2+}. Regulation of gene expression is involved in the origin of circadian rhythms and of the segmentation clock that controls somitogenesis.

A common property of regulatory feedback loops is their capability of inducing instabilities. Of relevance to rhythmic behavior is the situation where the regulated system undergoes sustained oscillations around a steady state that has become unstable beyond a critical value of some control parameter or in a parameter range bounded by two critical values [1,2]. For example, intracellular Ca^{2+} oscillations occur in many cell types subjected to intermediate levels of hormonal stimulation. Below a critical value of stimulus intensity, the level of Ca^{2+} stabilizes at a low constant level, while above a second, higher critical value, cytosolic Ca^{2+} reaches a high steady-state level. Likewise sustained oscillations occur in glycolysing yeast extracts in a range bounded by two critical values of the substrate input rate. Because they occur beyond a critical point of instability of a nonequilibrium steady state, biological rhythms can be viewed as temporal dissipative structures [3].

A flurry of cellular rhythms have been discovered during the last decade. Among these is the segmentation clock which controls the periodic formation of somites in vertebrate development. This system is of particular interest because of its key role in embryogenesis and the fact that it records a temporal structure as a permanent pattern of spatial organization [4]. The clock is also expressed in cell cultures in the form of oscillatory transcription of the gene *Hes1* [5]. Other recently observed rhythms include oscillations in both the tumor suppressor p53 [6] and the transcription factor NFκB [7], stress-induced oscillations in the nucleocytoplasmic shuttling of the transcription factor Msn2 in yeast [8], and the periodic organization of the yeast transcriptome [9].

Because rhythmic behavior cannot be ascribed to a single gene or enzyme, and rather constitutes a systemic property originating from regulatory interactions between coupled elements in a metabolic or genetic network, cellular rhythms represent a prototypic field of research in systems biology. Models help unraveling the dynamics of cellular rhythms and show that sustained oscillatory behavior often corresponds, in the concentration space, to the evolution toward a closed curve known as a limit cycle. Cycling once around this trajectory takes exactly one period. The closed trajectory is generally unique in a given set of conditions, and is particularly stable as it can be reached regardless of initial conditions. The limit cycle is a central figure in the study of biological rhythms [1,2].

The major questions regarding cellular rhythms pertain to their molecular mechanism and to their physiological function. With regard to mechanism, an oscillation can be divided into a succession of phases: understanding the mechanism of the rhythm amounts to clarifying how one phase brings about the next one, and why the process is repetitive — why the first phase is necessarily induced by the last phase of the preceding cycle. This analysis is facilitated by the use of models that present the advantage of allowing us to freeze the behavior of an oscillatory system at any given moment over the period,

Table 1. The main biological rhythms.

Biological rhythm	Period
Neural rhythms*	0.001 to 10 seconds
Cardiac rhythm*	1 second
Calcium oscillations*	Seconds to minutes
Biochemical oscillations*	30 seconds to 20 minutes
Mitotic oscillator*	10 minutes to 30 hours
Segmentation clock*	30 minutes to 2 hours
Hormonal rhythms*	10 minutes to 3–5 hours (also 24 hours)
Circadian rhythms*	About 24 hours
Ovarian cycle	28 days (menstrual cycle)
Annual rhythms	1 year
Rhythms in ecology and epidemiology	Years

*These rhythms can already occur at the cellular level.

so as to clarify the relationships between the variables and the process that predominates at any particular phase of the cycle.

The molecular bases of periodic behavior have been particularly well studied in the case of circadian rhythms. In eukaryotic organisms, these rhythms appear to originate from indirect, negative autoregulation of gene expression (see [10] and the reviews devoted to circadian rhythms in this issue). The mechanism of the circadian clock differs in cyanobacteria, where it involves a cyclical process of protein phosphorylation [11]. The segmentation clock involves a network of coupled signaling pathways, each of which appears to be capable of oscillations likely due to negative feedback [12,13]. Negative regulation can be mediated by repression of gene transcription, activation of protein degradation, or inhibition of a kinase or phosphatase in a signaling pathway through reversible phosphorylation. Negative feedback on transcription is also at the core of the 'repressilator', a synthetic gene oscillatory network based on three cyclically coupled repressors [14].

Although all biological rhythms reflect a process of temporal self-organization, some do not serve directly to measure the passage of time. Thus, rhythms that link the periodic generation of action potentials to muscular movement underlie the periodic operation of physiological machines: the cardiac rhythm ensures the periodic contraction of the heart, while oscillatory neural networks known as central pattern generators drive the rhythms of locomotion and respiration. Many biological rhythms, however, do have a function associated with the measure of time. Adaptation to the periodic nature of the terrestrial environment is the role of the circadian clock and of annual rhythms. Thus, oscillations of 24-hour period allow the organisms to adapt to and anticipate the alternation of day and night. Likewise annual rhythms underlie adaptation to the cycle of the seasons. The circadian clock and seasonal rhythms provide the strongest links between biological periodicities and time.

Circadian and annual rhythms are often closely intertwined, as shown by the example of flowering: the annual rhythmic variation of the photoperiod — which measures the duration of the light phase over 24 hours — controls the phase of the plant circadian clock. The expression of genes leading to flowering is triggered when the circadian peak in a particular protein coincides with the light phase. Another example of the close link between the circadian and annual clocks is given by the migratory behavior of the Monarch butterfly. Elegant experiments have shown that the butterfly uses the phase of its circadian clock to select orientation during flight [15].

The link between biological rhythms and time can take additional forms. An important function of oscillations is to allow for the frequency encoding of pulsatile signals in intercellular communication. One of the most striking examples is given by the pulsatile secretion of gonadotropin-releasing hormone (GnRH). This hormone is released by the hypothalamus with a frequency of one pulse per hour. The frequency of the hormone governs its physiological efficiency: a less or more frequent pulsatile release fails to elicit the secretion of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) by the pituitary at levels required for ovulation [16]. Such a frequency encoding of the pulsatile signal is also observed for intercellular communication by pulses of cyclic AMP in *Dictyostelium* amoebae. In this case, as in the case of GnRH, receptor desensitization under constant stimulation provides a basis for the frequency encoding of the pulsatile signal. The kinetics of receptor desensitization and resensitization determine the optimal frequency that ensures maximum target cell responsiveness [1]. A similar explanation holds for growth hormone signaling, which proves more efficient when delivered in pulses at the optimal frequency rather than in a continuous manner.

Frequency encoding is also encountered for intracellular pulsatile signaling, as observed for oscillations of cytosolic Ca^{2+}. The waveform and frequency of Ca^{2+} signals can exert profound effects on gene expression, and can thereby modulate neuronal differentiation [17]. One mechanism for encoding of Ca^{2+} spikes relies on the control of Ca^{2+}-activated enzymes such as CaM-Kinase II [18]. Signaling in the brain is also based on the frequency of trains of action potentials, which can be modulated by the intensity of neuronal stimulation [19]. The oscillatory activity of neurons appears to play a central role not only in transducing sensory responses but also in the neurobiology of consciousness.

Physiological disorders may arise from a mismatch between internal biological time and environmental cues. The clearest case pertains to the sleep–wake cycle. Hypophosphorylation of PER2, one of the major actors in the circadian clock mechanism, shortens the autonomous period of the clock and leads to a phase advance of several hours with respect to the natural light–dark cycle [20]. This alteration of circadian oscillations underlies the familial sleep phase advance syndrome (FASPS). Lack of entrainment can also occur, in the form of the non-24 hour sleep–wake syndrome by which the sleep-wake cycle runs at its own free-running period, unconnected with the light–dark cycle. Mood disorders also appear to be related to circadian rhythms [21] and may further possess an annual component, as reflected by seasonal affective disorders. Disorders related to dysfunctions of ultradian cellular oscillations are also known. Among these are syndromes associated with the absence of Ca^{2+} oscillations or waves, and cases of sterility resulting from alterations in the pulsatile secretion of the hormone GnRH.

The cell cycle itself may be viewed as a cellular rhythm, not only in the case of early cycles in frog embryos but also in more complex cell cycles subjected to checkpoints in somatic cells. Such a view leads to considering cancer as a dysfunction of normal cyclical or non-cyclical behavior of the biochemical machinery controlling cell proliferation. Cross-talk between different rhythms is illustrated by the link between the cell cycle and the circadian clock [22]. The latter controls the expression of the kinase wee1, thereby inhibiting in a circadian manner the kinase Cdk1 and, hence, the G2/M transition.

From a clinical perspective, the relationship between rhythms

and time in biological systems bears on the appropriate timing of medications. Experimental and clinical evidence shows that the circadian administration of anticancer drugs at the right time of the day can prove more efficient against tumor cells while minimizing damage to host tissues. Such chronotherapeutical approaches are based on the observation that many cellular processes, including those controlling cell proliferation or the activity of drug-degrading enzymes, vary in a circadian manner [23].

The ubiquity and physiological significance of biological rhythms can be illustrated by one last example, which shows how rhythms are often nested in a manner reminiscent of Russian dolls. In the process of reproduction, several rhythms play key roles at different stages and with markedly distinct periods. Fertilization of an egg triggers a train of Ca^{2+} spikes that are essential for successful initiation of development. Prior to these Ca^{2+} oscillations of a period of the order of minutes, ovulation requires appropriate levels of LH and FSH established through pulsatile signaling by GnRH with a period close to one hour (the response of pituitary cells to GnRH also involves high-frequency Ca^{2+} oscillations). The ovulation cycle is itself periodic, and takes the form of the menstrual cycle in the human female. Capping these various periodicities, in many animal species reproductive activity varies according to an annual rhythm controlled by the photoperiod, through modulation of the circadian secretion of melatonin [24]. In a final manifestation of the ticking of the biological clock, ovulation stops at menopause. At the very core of life, the reproductive process highlights the deeply rooted links between rhythms and time in biological systems.

References

1. Goldbeter, A. (1996). Biochemical Oscillations and Cellular Rhythms (Cambridge, UK: Cambridge University Press).
2. Goldbeter, A. (2002). Computational approaches to cellular rhythms. Nature *420*, 238–245.
3. Goldbeter, A. (2007). Biological rhythms as temporal dissipative structures. Adv. Chem. Phys. *135*, 253–295.
4. Pourquié, O. (2003). The segmentation clock: Converting embryonic time into spatial pattern. Science *301*, 328–330.
5. Hirata, H., Yoshiura, S., Ohtsuka, T., Bessho, Y., Harada, T., Yoshikawa, K., and Kageyama, R. (2002). Oscillatory expression of the bHLH factor Hes1 regulated by a negative feedback loop. Science *298*, 840–843.
6. Lahav, G., Rosenfeld, N., Sigal, A., Geva-Zatorsky, N., Levine, A.J., Elowitz, M.B., and Alon, U. (2004). Dynamics of the p53-Mdm2 feedback loop in individual cells. Nat. Genet. *36*, 147–150.
7. Hoffmann, A., Levchenko, A., Scott, M.L., and Baltimore, D. (2002). The IkappaB-NF-kappaB signaling module: Temporal control and selective gene activation. Science *298*, 1241–1245.
8. Garmendia-Torres, C., Goldbeter, A., and Jacquet, M. (2007). Nucleocytoplasmic oscillations of the yeast transcription factor Msn2: evidence for periodic PKA activation. Curr. Biol. *17*, 1044–1049.
9. Tu, B.P., Mohler, R.E., Liu, J.C., Dombek, K.M., Young, E.T., Synovec, R.E., and McKnight, S.L. (2007). Cyclic changes in metabolic state during the life of a yeast cell. Proc. Natl. Acad. Sci. USA *104*, 16886–16891.
10. Reppert, S.M., and Weaver, D.R. (2002). Coordination of circadian timing in mammals. Nature *418*, 935–941.
11. Nakajima, M., Imai, K., Ito, H., Nishiwaki, T., Murayama, Y., Iwasaki, H., Oyama, T., and Kondo, T. (2005). Reconstitution of circadian oscillation of cyanobacterial KaiC phosphorylation in vitro. Science *308*, 414–415.
12. Dequeant, M.L., Glynn, E., Gaudenz, K., Wahl, M., Chen, J., Mushegian, A., and Pourquié, O. (2006). A complex oscillating network of signaling genes underlies the mouse segmentation clock. Science *314*, 1595–1598.
13. Goldbeter, A., and Pourquié, O. (2008). Modeling the segmentation clock as a network of coupled oscillations in the Notch, Wnt and FGF signaling pathways. J. Theor. Biol. *252*, 574–585.
14. Elowitz, M.B., and Leibler, S. (2000). A synthetic oscillatory network of transcriptional regulators. Nature *403*, 335–338.
15. Froy, O., Gotter, A.L., Casselman, A.L., and Reppert, S.M. (2003). Illuminating the circadian clock in Monarch butterfly migration. Science *300*, 1303–1305.
16. Belchetz, P.E., Plant, T.M., Nakai, Y., Keogh, E.J., and Knobil, E. (1978). Hypophysial responses to continuous and intermittent delivery of hypothalamic gonadotropin-releasing hormone. Science *202*, 631–633.
17. Spitzer, N.C., Lautermilch, N.J., Smith, R.D., and Gomez, T.M. (2000). Coding of neuronal differentiation by calcium transients. BioEssays *22*, 811–817.
18. De Koninck, P., and Schulman, H. (1998). Sensitivity of CaM kinase II to the frequency of Ca^{2+} oscillations. Science *279*, 227–230.
19. Buzsaki, G., Llinas, R., Singer, W., Berthoz, A., and Christen, Y., eds. (1994). Temporal Coding in the Brain (Berlin: Springer).
20. Toh, K.L., Jones, C.R., He, Y., Eide, E.J., Hinz, W.A., Virshup, D.M., Ptacek, L.J., and Fu, Y.H. (2001). An hPer2 phosphorylation site mutation in familial advanced sleep phase syndrome. Science *291*, 1040–1043.
21. McClung, C.A. (2007). Circadian genes, rhythms and the biology of mood disorders. Pharmacol. Ther. *114*, 222–232.
22. Matsuo, T., Yamaguchi, S., Mitsui, S., Emi, A., Shimoda, F., and Okamura, H. (2003). Control mechanism of the circadian clock for timing of cell division in vivo. Science *302*, 255–259.
23. Levi, F., and Schibler, U. (2007). Circadian rhythms: mechanisms and therapeutic implications Annu. Rev. Pharmacol. Toxicol. *47*, 593–628.
24. Malpaux, B., Migaud, M., Tricoire, H., and Chemineau, P. (2001). Biology of mammalian photoperiodism and the critical role of the pineal gland and melatonin. J. Biol. Rhythms *16*, 336–347.

Faculté des Sciences, Université Libre de Bruxelles (ULB), Campus Plaine, CP 231, B-1050 Brussels, Belgium.
E-mail: agoldbet@ulb.ac.be

Quick guide

Circatidal clocks

David Wilcockson[1,*] and Lin Zhang[2]

Tides and tidal clocks. Circadian clocks allow terrestrial organisms to coordinate their behaviour and physiology to the relentless 24 hour rhythms of day and night. In contrast, residents of coastal or estuarine habitats, including crustacea, annelids, molluscs, fish and even a few insects, modulate their behaviour in tune to the ~12.4 hour ebb and flow of the tides. On most coastlines of the world, high and low tides occur twice in each solar day with an interval of about 12.4 hours. Moreover, the relative amplitude of the tides alters gradually over the course of the lunar month, so that about every 15 days there are the semi-lunar maximal spring tides when the sun, earth and moon become aligned at new and full moons, and the minimal neap tides, when the moon is at 90° to the earth, relative to the sun (Figure 1). Thus, the predictable inundation and exposure of the intertidal zone brings about rapid changes in salinity, temperature, hydrostatic pressure, turbulence and food availability that challenge the inhabitants of this ecosystem with a complex mixture of cycling environmental stimuli.

Tidal locomotor behaviour. Crustacea have provided the favoured model systems in tidal research. Fiddler crabs (*Uca pugnax*) live in burrows along shores and emerge at low tide to forage, mate and fight. In contrast, the ubiquitous green shore crabs, *Carcinus maenas*, prefer to remain hidden under rocks or weeds on the mid-shore, until the tide covers their foraging and mating grounds, when they become active. When removed to the laboratory and held in constant conditions, locomotor activity bouts of both crab species continues at times of expected low water (fiddler) or high water (green crab) with a 12.4 h interval between peaks. This free running behaviour indicates the presence of tidal clocks in these animals, which under natural conditions would be synchronised to the phase of the tidal cycle encountered on their home beach.