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.

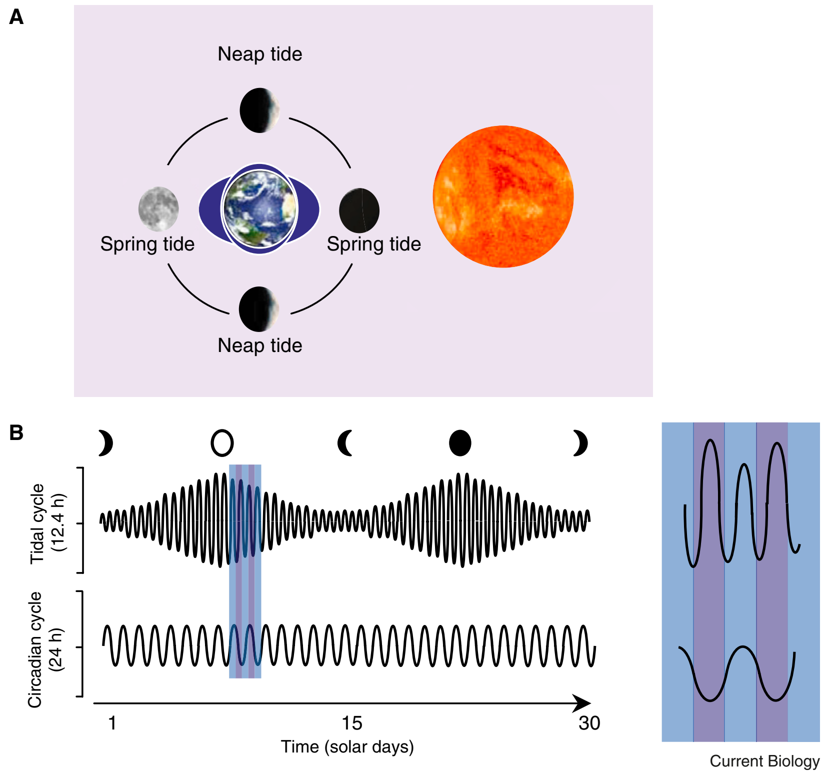


Figure 1. Spring and neap tides.

(A) The gravitational pull of the moon and sun, together with the rotation of the earth and moon and sun, pull water masses into bulges on each side of the earth nearest and furthest from the moon. This movement of the earth's water masses (shaded blue around the earth) produces the tides which, on most coasts occur twice per solar day. Each tidal cycle takes 12.4 hours to complete. When the earth, moon and sun are aligned, at new and full moons, their combined gravitational forces create greater tidal range: these are known as spring tides. When the moon is at right angles relative to the sun and earth, at the first and third quarters of the moon, gravitational pull is at its weakest and the tidal range is small, the so-called neap tides. (B) The relative amplitude of spring and neap tides. During the course of the lunar month the alternation between spring and neap tides occurs twice at ~15 day intervals. Because of the inclination of the earth relative to the moon, the tidal range is unequal between successive high tides. The expanded view (blue panels) shows the spring tidal amplitude is maximal at night time (dark blue panels) high water. These cartoons demonstrate a rather simple scenario. In reality of course the situation is more complex and there may be considerable local differences in tidal regimes.

Smaller crustacea, such as the cirolanid isopod *Eurydice pulchra* from the northwest Atlantic, exhibit robust circatidal swimming rhythms in constant conditions that are more easily monitored in the laboratory (Figure 2). In the wild, these creatures live buried under the sand at around the high water mark. As the incoming tide advances, they are disinterred from the sand by wave action and swim for several hours in the wave margins where they feed and mate, returning to the sand before the tide recedes. In this way, they maintain their preferred position on the shore and avoid being stranded above the high water mark on large amplitude, spring tides or swept into deep water.

Intertidal insects are not common and their circatidal rhythms usually persist for a only a few days. In contrast, the Asian mangrove cricket, *Apteronemobius asahinai*, shows robust tidal cycles by foraging on the muddy swamp floor during low water until the incoming tide forces them to climb back into the limbs of the mangroves to avoid drowning and predation. In the laboratory, divorced from such cues, the tidal patterns of behaviour persist. Their locomotor activity peaks during night-time high tides — or, in constant conditions, at the expected night-time high tides — revealing a circadian modulation of the underlying tidal activity, something also observed with crabs and *Eurydice pulchra*. Thus, field collections of *Eurydice pulchra* are most successful during night-time high water on spring tides, when swimming is maximal, compared to neap tides, when fewer animals emerge from the sand. Under laboratory conditions that use cycles of artificial water turbulence, tidal swimming is enhanced by simultaneous light–dark cycles when the dark phase coincides with the shaking, as would happen in nature on high water spring tides.

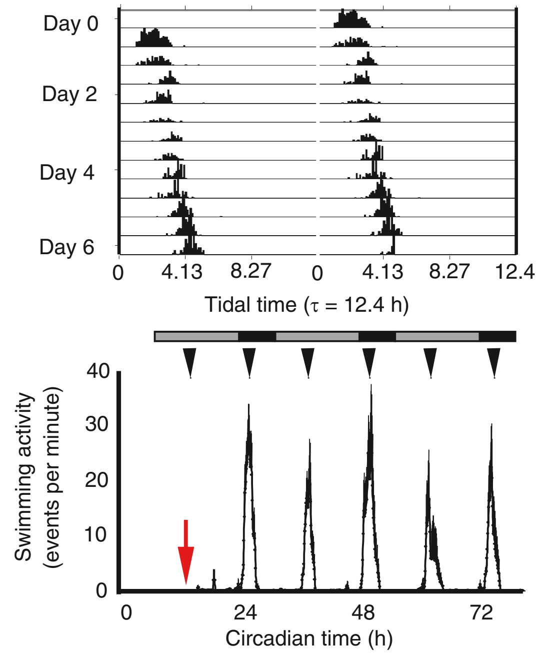
Tidal life history traits. As well as circatidal locomotor behaviour, some organisms use tides to coordinate reproduction with local tidal regimes; this is true, for example, of chironomid midges such as *Clunio marinus*. Adult flies deposit their eggs on seaweed fronds around the low water mark on spring tides where the larvae develop, feeding on detritus. When larval development is complete, eclosion takes place at low water on subsequent spring tides. This faithful tidal and semilunar behaviour ensures reproductive success. Under laboratory conditions, eclosion rhythms have been entrained by subjecting larvae to cycles of artificial moonlight. It has been proposed that lunar cues govern the time of month at which to emerge, whilst the specific hours of emergence are gated according to a circadian clock.

Numerous crustacean species are known to use tidal flow in order to aid the dispersal of larvae. For example, female *Carcinus maenas* and *Uca pugilator* release their eggs in estuaries about the time that the outgoing (ebbing) tidal flow is maximal. After hatching, the larvae swim upwards each day as the tide ebbs and return to the seabed when the tide reverses and pushes upriver. This endogenously timed activity results in the net movement of the larvae towards the open sea where conditions are favourable for growth and metamorphosis. Crabs taken from the shore and kept in constant conditions continue to behave in parallel to those from where they were spawned. It is possible that the release of the larvae during the ebb tide is the initial zeitgeber that sets the larval tidal clocks in motion.

How are tidal rhythms generated? Two mechanisms underlying tidal rhythms have been proposed. The 'circalunidian clock hypothesis' is based on the lunar day (24.8 hours) and asserts that two, loosely coupled lunidian oscillators run in antiphase to produce 12.4 hour intervals. This notion is supported by the observation that the circatidal locomotor activity peaks of intertidal crabs kept in constant conditions may drift or even temporarily disappear before reappearing on the next cycle, a scenario unlikely to occur with a dedicated circatidal oscillator. In some cases, daily behaviour may be unimodal or bimodal depending on the stimulus received. For example, the bimodal circatidal behaviour of crabs from diurnal tidal regimes becomes unimodal when the animals are translocated to shores which have only one tide per day. Proponents of the circalunidian hypothesis argue also that it represents the most economical answer to the basis of tidal rhythmicity. It has even been suggested that true tidal clocks are not unequivocally distinguishable from circadian clocks. Terrestrial organisms undoubtedly possess multiple clocks. In *Drosophila* distinct neuronal groups govern independent activity bouts phased to morning or evening and so are roughly 12 hours apart depending on photoperiod. In hamsters kept in constant light, their locomotor activity can 'split' into two components 12 hours apart that reflect antiphase oscillations of key clock gene transcripts in each half of the master clock (suprachiasmatic nucleus). These observations may provide a precedent for the circalunidian model but, how would such a circalunidian timer generate lunar patterns?

The second school of thought advocates that dedicated circatidal clocks with a period of about 12.4 hours are the basis for tidal behaviour. Superimposed on this tidal clock is a circadian oscillator that drives the day/night modulation observed in locomotor output of crabs, crickets and isopods (Figure 2). There are several lines of evidence to support this idea. In the green shore crab, it has been shown experimentally that separate circadian and circatidal rhythms can be induced. The behaviour of marine midges has been shown to have a circadian and tidal component and it is now generally accepted that tidally rhythmic animals are guided by multiple endogenous oscillators. The discovery that crabs can be entrained by different, artificial tidal 'cues', such as changes in hydrostatic pressure, temperature and salinity, have led to careful behavioural analysis demonstrating that clocks can be 'split' if cues are delivered out of phase to each other.

Eurydice pulchra



Current Biology

Figure 2. Tidal clocks.

The intertidal isopod *Eurydice pulchra* (left) shows rhythmic swimming activity that coincides with high tide. When removed from the wild into the laboratory the swimming activity persists for several days and matches the time of expected high water on the beach from where the animals were collected. The actograph (top right) shows the activity recorded from a single animal held in constant dark after being taken from a beach in North Wales. The histogram (bottom right) illustrates the mean behaviour of ten individuals taken from the same beach over five complete tidal cycles. Black arrows indicate the time of expected high water and the red arrow shows when the animals were captured. The black and grey bars indicate time of expected day and night. Note that the amplitude of alternate activity peaks modulates according to expected night time high water when the tidal range is greatest.

Looking ahead. No one model can fully explain all the observed nuances of tidal animals. To date, research on tidal rhythms has relied mainly upon elegant behavioural analysis, with very limited insight into the cellular or indeed physiological basis of the corresponding oscillators. The molecular basis of circatidal rhythms has received scant attention compared to the major effort (and remarkable advances) made in circadian biology. Using contemporary molecular techniques, the phenotypes of tidally rhythmic animals can now be interrogated at their cellular source and some effort is being directed into producing genomic and transcriptomic tools for non-model marine species. Notwithstanding their intrinsic interest or their ecological and in some cases economic importance, marine animals represent a significant component of global biodiversity, so understanding their adaptive timing mechanisms is of considerable importance. Moreover, marine animals pre-date their terrestrial relatives and the question arises as to whether circadian clocks could have originally evolved from tidal oscillators?

Where can I find out more?

De la Iglesia, H.O., Meyer, J., Carpino, A. Jr., and Schwartz, W.J. (2000). Antiphase oscillation of the left and right suprachiasmatic nuclei. Science *290*, 799–801.

Grima, B., Chélot, E., Xia, R., and Rouyer, F. (2004). Morning and evening peaks of activity rely on different clock neurons of the *Drosophila* brain. Nature *431*, 869–873.

Hastings, M.H. (1981). The entraining effect of turbulence on the circa-tidal activity rhythm and its semi-lunar modulation in *Eurydice pulchra*. J. Mar. Biol. Ass. UK *61*, 151–160.

Naylor, E. (1996). Crab clockwork: The case for interactive circatidal and circadian oscillators controlling rhythmic locomotor activity of *Carcinus maenas*. Chronobiol. Int *13*, 153–161.

Palmer, J.D. (2000). The clocks controlling the tide-associated rhythms of intertidal animals. BioEssays *22*, 32–37.

[1]School of Biological Sciences, Bangor University, Wales, UK. [2]Department of Genetics, Leicester University, UK.
*E-mail: d.wilcockson@bangor.ac.uk