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Review

Clocks at sea: the genome-editing tide is rising

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The coastline is a particularly challenging environment for its inhabitants. Not only do they have to cope with the solar day and the passing of seasons, but they must also deal with tides. In addition, many marine species track the phase of the moon, especially to coordinate reproduction. Marine animals show remarkable behavioral and physiological adaptability, using biological clocks to anticipate specific environmental cycles. Presently, we lack a basic understanding of the molecular mechanisms underlying circatidal and circalunar clocks. Recent advances in genome engineering and the development of genetically tractable marine model organisms are transforming how we study these timekeeping mechanisms and opening a novel era in marine chronobiology.

Highlights

Marine organisms track multiple environmental cycles with dedicated biological clocks and thus show remarkably plastic molecular and behavioral rhythms.

The combination of new marine model organisms and genome-engineering technologies are advancing our understanding of the molecular mechanisms underlying circatidal and circalunar rhythms.

Brain and muscle Arnt-like protein-1 (BMAL1) is a molecular link between the circadian (24 h) and circatidal (12.4 h) clocks, while PERIOD and CRYPTOCHROME 2 appear to be specific to circadian rhythms.

Specific opsin and cryptochrome photoreceptors allow organisms to recognize moonlight and adjust their rhythmic behavior accordingly.

Biological rhythms in marine organisms

For a chronobiologist, the coastline is a fascinating ground for scientific exploration. Within it, organisms of all kinds use biological clocks to keep track of diverse environmental cycles: the solar day, the tides, the phase of the moon, and even the seasons (Figure 1). Commonly, the inhabitants track more than one cycle simultaneously. Over the past 100 years, physiological and behavioral observations have demonstrated the unambiguous existence of biological clocks in marine organisms [1], but explaining them on a mechanistic level has been an immense challenge. What are the basic elements of the different endogenous clocks? How do they control behavior and physiology in a plastic fashion? Although these questions were once largely intractable, novel genome-editing approaches and the development of powerful marine model systems are now revolutionizing the field of marine chronobiology.

The intertidal zone is defined as the area of the coastline that can either be submerged or exposed by tides, creating a challenging niche for survival [2,3]. Like most life forms on Earth, intertidal animals must cope with the daily 24-h day/night cycle, but in addition they need to be capable of anticipating 12.4-h tidal rhythms in their environment. The tides bring oscillations in water height, turbulence, salinity, and temperature – defining physical characteristics of the environment that arguably impact physiology and behavior even more profoundly than the alternation of day and night with its photic and thermal cycles [4]. When brought into the laboratory and studied under constant conditions, intertidal organisms can exhibit both circadian and circatidal rhythms [5–9]. Thus, they must possess distinct circadian and circatidal molecular clocks. Interestingly, tidal rhythms have been observed in organisms from beyond the intertidal zone in the adjacent subtidal zone and, astoundingly, even near deep-sea thermal vents, as abiotic factors such as water temperature, currents, and water height, as well as food availability, might be tidally rhythmic even under constant submersion [10–12]. The phase of the lunar month (colloquially called the 'phase of the moon') also impacts the behavior and physiology of many marine organisms, particularly in relation to their reproduction [13]. Some of the most spectacular rhythmic events in nature are linked to moon phase, such as the coordinated coral spawning in the Great Barrier Reef and the Christmas Island crab migration. As with their circatidal counterparts, circalunar (~29.5 day) reproductive rhythms can be observed even under constant laboratory conditions [14].

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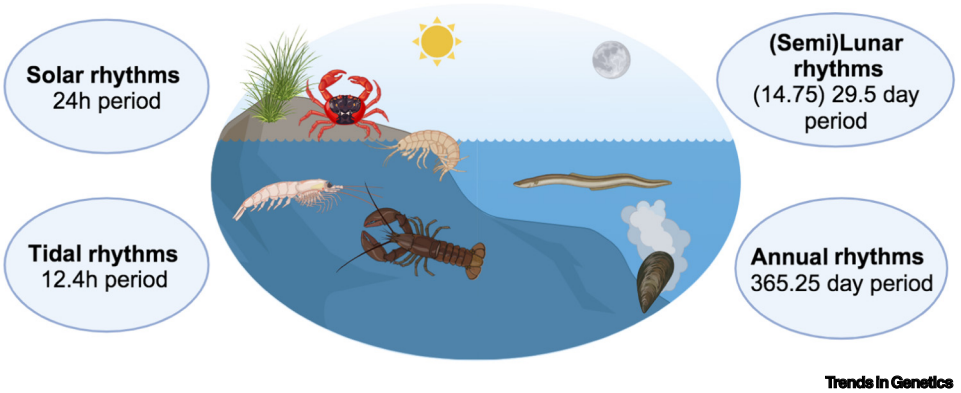

Figure 1. Organisms living in the intertidal zone and beyond are exposed to a variety of environmental cycles with different periodicities.

The existence of multiple biological clocks in the same animal is, as mentioned earlier, well documented. Interestingly, some animals can switch their behavioral or gene expression rhythms between circadian and circatidal patterns depending on environmental conditions [15,16] or remarkably, in Cnidaria, based on the presence or absence of symbionts [17]. Mixed behavioral patterns can also be observed, with one clock modulating the behavior driven by another [9,17–20]. This behavioral and molecular plasticity is probably required to deal with the complexity of the intertidal environment. For example, in the Red Sea limpets' tidal behavior showed pronounced daily modulation specifically during the months of September and October, when overall water levels are higher [18]. In addition to seasonal factors, the amplitude of tides varies during the lunar month, with spring tides occurring at new and full moon and neap tides at half-moons. While the Moon has a dominant effect on tides, the total tidal force experienced on Earth is at its highest when the Sun, Moon, and Earth are aligned (Figure 2). For an animal living in a shallow mangrove swamp, this might result in periods of constant submersion or exposure. During these periods, it might make sense for circadian rhythms to drive behavior and physiology, while the circatidal clock would dominate during periods of rhythmic submersion.

Interactions between different clocks can also be used to overcome specific challenges. A striking and well-studied example is that of the coastal midge *Clunio marinus*. This dipteran insect from the European Atlantic coastline, whose terrestrial adult stage is limited to a few hours, must reproduce during the lowest possible tide so that females can lay eggs on dry substrate that will then be continuously submerged for the remainder of the semilunar cycle, allowing the embryos and larvae to develop in an aqueous environment. Importantly, in a given location, the lowest tide always occurs at the same time of the day on either the new or the full moon. How do they predict the lowest tide? The logical evolutionary solution, which midges use, is to combine a circalunar clock (or more precisely a circa-semilunar clock, ~14.7 days; see later) that tracks the phase of the lunar month to regulate the timing of development with a circadian clock that determines the precise time of day at which adults emerge and reproduction can occur [3,21,22]. However, there is an additional challenge for these midges: the phase of tides varies considerably from location to location because of the shape of the coastline and of the ocean floor. Thus, in one location, the lowest tide might always occur at 14.00 h on full or new moons, while at a second location it might occur at 18.00 h. Midges isolated from different locations show different phases of adult emergence, and thus of reproduction, adjusted to the timing of their local tides, and these persist even in a laboratory setting [21,23,24]. These temporal traits are genetically encoded, and genomic studies on geographically separated *Clunio* isolates have provided interesting gene variants that might determine emergence

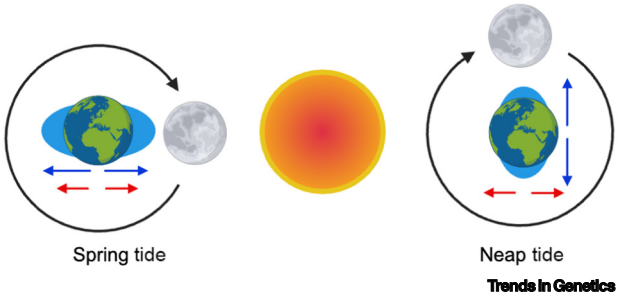

Figure 2. Alignment of the Moon, Earth, and Sun impacts the amplitude of tides. When the tidal forces caused by the Sun and Moon are aligned (red and blue arrows, respectively) at new or full moons, the amplitudes of tides on Earth are maximal (left). When the tidal forces are perpendicular, lower-amplitude neap tides are generated (right).

phase, in particular in the gene encoding calmodulin-dependent kinase II (CAMKII)) [23]. Multiple lines of evidence, correlative or in heterologous systems, indicated that a *CamKII* polymorphism impacts its splicing pattern and thus CAMKII activity. However, a direct demonstration that the *CamKII* polymorphism impacts the phase of *Clunio*'s emergence is still missing. This is unsurprising. Midges, and marine organisms in general, are not easily manipulated genetically. In addition, propagation and entrainment in the laboratory can be challenging, if not impossible. Nevertheless, the molecular study of marine biological clocks is accelerating, fueled by transcriptome-wide gene expression studies, new genome-editing approaches, and the development of promising new laboratory model systems.

Marine circadian clocks

Circadian clocks are by far the best understood biological timers. In animals, circadian rhythms are generated by a transcriptional feedback loop comprising transcriptional activators promoting the expression of their own repressors [25,26]. Additional layers of control, such as post-translational modifications, protein degradation, and secondary transcriptional feedback loops, create a stable *ca* 24-h period [25,27,28] (Figure 3). The overall result of this complex system, predominantly studied in *Drosophila* and mice, is the production of two main waves of transcription, at dawn and dusk. The core circadian transcriptional loop is highly conserved between *Drosophila* and mice [25,26,29]. In both species, a dimeric transcription factor comprising CLOCK (CLK)

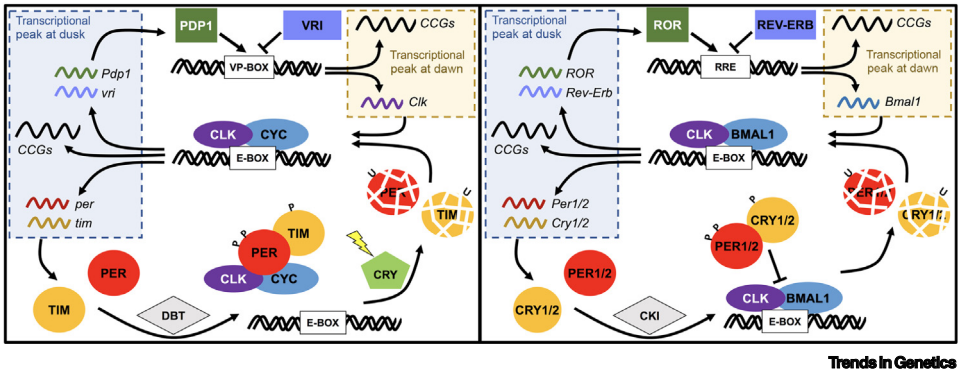

Figure 3. *Drosophila* (left) and mammalian (right) circadian clocks comprise two transcriptional feedback loops. Broken shapes and Us indicate that PERIOD (PER)1/2 and TIMELESS (TIM)/CRYPTOCHROME (CRY)1/2 are ubiquitinated and degraded following their repression of CLOCK (CLK) and CYCLE (CYC)/brain and muscle Arnt-like protein 1 (BMAL1). Lightning bolt in the *Drosophila* clock represents CRY's activation by blue/UV light. Abbreviations: *CCG*, circadian controlled gene; CKI, casein kinase I; DBT, DOUBLETIME; *Pdp1*, *PAR-domain protein 1*; *per, period; ROR, retinoic acid receptor-related orphan receptor; tim, timeless; vri*, *vrille*.

and brain and muscle Arnt-like protein-1 (BMAL1) [or CYCLE (CYC) in fruit flies] promotes the expression of their own repressors (Figure 3). In *Drosophila*, PERIOD (PER) and TIMELESS (TIM) interact to repress CLK/CYC transcription, while in mammals two PER homologs (PER1 and PER2) with two CRYPTOCHROME family members (CRY1/2) repress CLK/BMAL1 transcription. Homologs of these genes are normally found in marine organisms such as crustaceans, mollusks and insects, with a few exceptions. Surprisingly, PER has been lost in echinoderms [30], while neither PER nor TIM is present in cnidarians such as *Nematostella vectensis* and *Acropora digitifera* [31,32]. Studies of the molecular mechanism of the circadian clock in these marine organisms should lead to interesting insights into the evolution of circadian clocks.

Transcriptomic and genomic resources are greatly simplifying the identification of candidate circadian genes. Recently, many crustacean clock genes have been identified (e.g., [33,34]; J. Hunt, PhD thesis, University of Leicester, 2016). As in insects [35], there seem to be interesting variations in the way circadian clocks are built. For example, the Antarctic krill *Euphausia superba* possesses both TIM1 and CRY1, which are homologs of *Drosophila* TIM and CRY, respectively [34]. *Drosophila* CRY functions as a cell-autonomous circadian light sensor that triggers TIM degradation after light exposure to reset the clock (Figure 3) [36–38]. *Parhyale hawaiensis*, an amphipod crustacean, possesses neither TIM nor a light-sensitive CRY homolog [33], while the isopod *Eurydice pulchra* carries a TIM homolog but no light-sensitive CRY [9]. By contrast, the transcriptional repressor CRY2 appears to be ubiquitously present in crustaceans [39]. Transcriptional assays in heterologous systems using the putative krill core clock proteins suggest a conserved circadian clock mechanism with CLK/BMAL1 functioning as activators and PER/TIM/CRY2 as repressors [34]. Curiously, several crustacean species appear to carry an unusually structured CLK protein that is missing the key PAS-B domain, which should be required for interaction with BMAL1/CYC [33], raising the possibility that a different bHLH/PAS domain protein replaces CLK in some crustaceans.

Few studies have tested the role of core circadian genes from marine organisms *in vivo*. RNAi has been used in both the crustacean *E. pulchra* [9,40] and the mangrove cricket *Apteronemobius asahinai* [41,42]. In the former, *per* was downregulated by abdominal injection of long double-stranded RNAs to cause RNAi. As a result, circadian *tim* mRNA and pigmentation rhythms were disrupted, although not completely eliminated [9]. The amplitude of these rhythms, as well as the circadian modulation of circatidal behavior, were also reduced by *Bmal1* and *cry2* knockdown [40]. These observations support a key role for PER, BMAL1, and CRY2 in *E. pulchra*'s circadian clock. In *A. asahinai*, either *per* or *Clk* double- stranded (ds)RNAs were injected, and this compromised the circadian modulation of locomotor behavior [41,42]. Recently, CRISPR/Cas9 was used to disrupt the *Bmal1* gene in *P. hawaiensis* [15], and circadian rhythms of behavior were severely disrupted in the mutant animals.

Circatidal clocks

The ability of animals to switch rhythmic patterns between 12.4-h and 24-h rhythms, and the fact that these two periodicities are almost harmonic, begs the question of how similar circadian and circatidal clocks are. Are they entirely distinct, do they share some common elements, or are they perhaps generated by a single, plastic clock?

The first evidence of the circatidal clock's existence came from observations of tidally rhythmic behavior in the marine worm *Symsagittifera roscoffensis* in the absence of tidal environmental cues [43]. Further investigation led by Naylor using the crab *Carcinus maenas* demonstrated that both circatidal and circadian regulation of activity rhythms could be observed and that they could be altered independently of one another [6,44]. These findings supported the idea that a 12.4-h

circatidal clock drives circatidal rhythms, distinct from the 24-h circadian clock [44,45]. However, additional behavioral studies led to two competing hypotheses (Figure 4A). By studying how rhythmic behavior of the isopod *Excirolana chiltoni* responded to tidal cues, Enright proposed that a tidally responsive circadian clock drives 12.4-h circatidal rhythms by adjusting its period to 24.8 h [46]. However, Palmer and Williams' investigation in the crabs *Helice crasse* and *Macrophthalmus hirtipes* revealed that the two (approximately daily) tidal peaks of activity

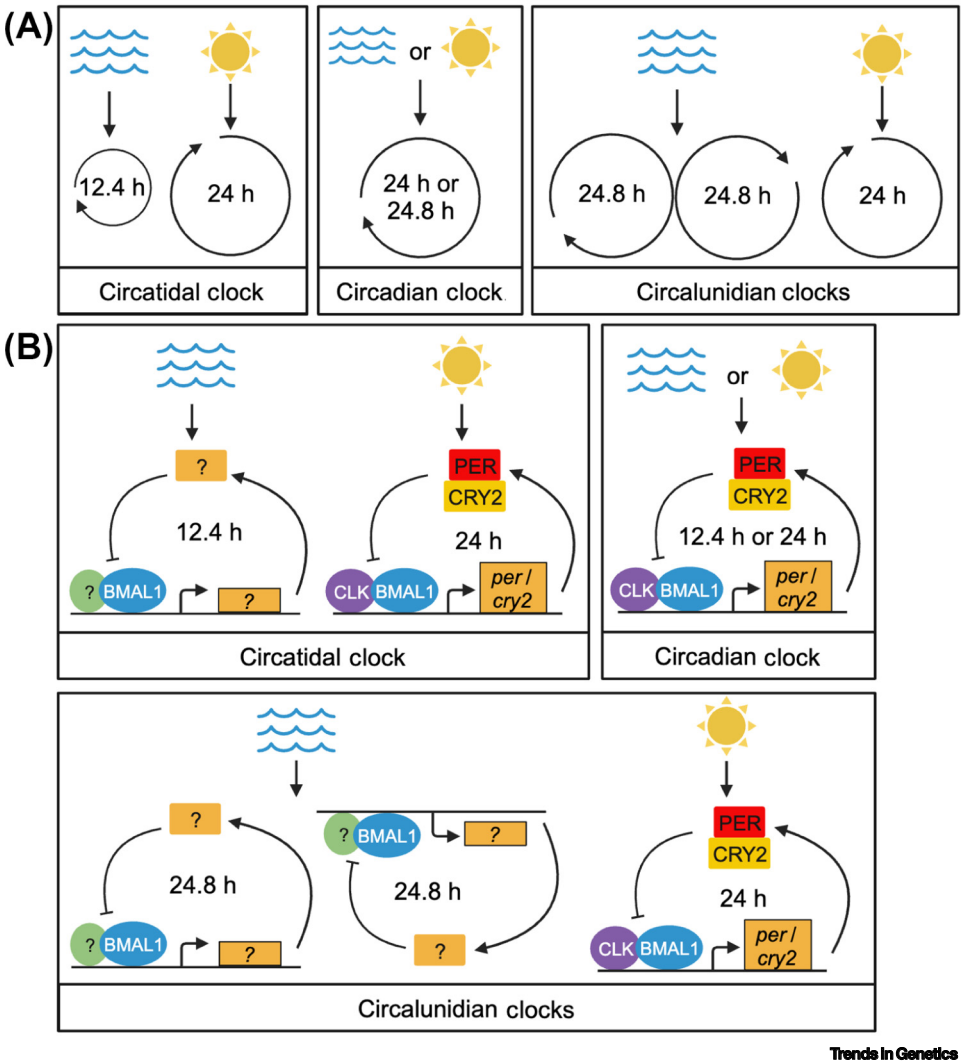

Figure 4. Circatidal clock models. (A) The three principal hypotheses for the circatidal clock. From left to right: The 12.4-h dedicated circatidal clock hypothesis proposed by Naylor [44,45], the flexible circadian clock hypothesis proposed by Enright [46], and the paired 24.8-h circalunidian clocks hypothesis proposed by Palmer and Williams [47,48]. (B) Simplified molecular models for the circatidal clock, corresponding to the three hypotheses shown in (A). The presence of both circadian and circatidal rhythms in several marine organisms, combined with current genetic evidence, supports the existence of distinct circatidal and circadian oscillators that share brain and muscle Arnt-like protein-1 (BMAL1) as a common core element. However, in some species, circadian clock gene expression can switch from 24 h to 12.4 h depending on environmental conditions. A plastic clock mechanism, similar to Enright's model but with a clock that can adopt either a 12.4-h or a 24-h rhythm, might thus be at play in some marine species. For simplicity, we used the circadian clock model for *Parhyale hawaiensis*, but as discussed in the main text, the repressors in the circadian clock vary between marine species.

appeared to be controlled separately, leading to the hypothesis that two circalunidian (24.8 h) clocks in antiphase work together to generate circatidal rhythms [47,48]. Interestingly, the molecular mechanism of the circadian clock, with its two antiphase waves of transcription (Figure 3), could be used as an antiphase double circalunidian molecular oscillator, with a minor period adjustment.

It is challenging to infer the molecular mechanisms driving the circatidal clock based solely on observations of behavioral outputs. Differing arrangements and hierarchies of signaling between circadian and circatidal pacemakers could be responsible for the behavioral patterns that led to the three hypotheses. It does seem likely that there is variability in hierarchies of signaling based on the variety of circadian–circatidal behavioral arrangements observed in nature. Many species exhibit circatidally rhythmic behavior that is modulated by the circadian clock (e.g., *E. pulchra* [9], *C. maenas* [44], *A. asahinai* [49]). The result is the expression of two peaks of activity with different amplitudes during the day and night. Some marine animals exhibit activity that is predominantly circadian but with circatidal modulation (*Dimorphostylis asiatica*) [50]. Finally, others express circatidal-dominant activity with little to no circadian modulation (*P. hawaiensis*) [15].

The most straightforward way to begin to distinguish between the three hypotheses shown in Figure 4A is to use genetics to target core circadian clock genes and evaluate the effect on circatidal behaviors. Consistent with the observations by Naylor and colleagues that circadian and circatidal rhythms could be manipulated separately [6,44], pioneer studies using RNAi suggested that the two clocks are distinct. Injection of dsRNAs against *A. asahinai per* [41] and *Clk* [42], as well as *E. pulchra per* [9], disrupted circadian rhythms as mentioned earlier. However, circatidal behavioral rhythms remained intact. By contrast, in *E. pulchra* pharmacological inhibition of the PER kinase CK1δ/ε reduced the amplitude of circadian rhythms and lengthened the period of tidal rhythms [9], suggesting that some elements might be shared between the two clocks. Since both the pharmacological and RNAi approaches have significant caveats such as off-targets and incomplete inhibition of activity and gene expression, the use of genetic knockouts is an appealing approach to yield more definitive answers. However, the creation of knockouts is not without its own potential drawbacks. A complete knockout could be lethal, and lifelong disruption of a given gene might have developmental or physiological consequences that extend beyond the gene's role in adulthood. Thus, a combination of gene knockouts and adult-specific RNAi is advantageous in reaching solid conclusions on gene function.

P. hawaiensis is a fully genetically tractable marine model organism that inhabits the intertidal zone [51], making it an ideal candidate to dissect the genetics of circatidal rhythms. Indeed, *P. hawaiensis* exhibits robust circatidal rhythms after entrainment to artificial tides [15]. Knockout of *Bmal1* using CRISPR/CAS9 demonstrated that this gene is required not only for circadian behavior but also for circatidal behavior. BMAL1 is thus a molecular link between the two clocks [15] (Figure 4B). Importantly, RNAi knockdown of *Bmal1* in *E. pulchra* also supports its role in the circatidal clock, but similar experiments for *per* and *cry2* had no impact on circatidal rhythms [40]. Thus, of the three core clock genes that have been tested so far, it appears that only *Bmal1* is shared between the two clocks (Figure 4B). Negative results using RNAi should however be taken with caution because of incomplete inactivation of expression. It will be important to corroborate RNAi results with gene knockout to determine definitely whether core circadian genes besides *Bmal1* are required for circatidal rhythms (see Outstanding questions). CLK is a particularly intriguing case, given that, based on its structure in several crustaceans, one would predict that it does not interact with BMAL1 and thus might be replaced by another bHLH/PAS protein [33]. HIF1 would be an interesting candidate since in mice it helps peripheral circadian clocks in sensing rhythmic changes in oxygen levels [52,53].

Rhythmic gene expression profiles can be used to identify core molecular clock components and could be a useful tool to identify the key players in the circatidal clock. Circadian transcriptomics, particularly in flies and mice, has been used to identify thousands of transcripts that oscillate with a 24-h pattern. Interestingly, core circadian clock genes oscillate in all tissues, while most rhythmic genes under their control are tissue specific [54–60]. Given these characteristics, circatidal transcriptomics might help to identify core circatidal genes, which should show a 12.4-h or perhaps a 24.8-h period (Figure 4; in the latter case, different tidal entrainment phases can be used to differentiate circadian and circatidal transcripts). Such studies have been performed in mussels, horseshoe crabs, limpets, and mangrove crickets [11,18,61–64], with most of these studies performed under entrained conditions (i.e., in the presence of environmental cycles such as day/night and tidal cycles). This is an important caveat, as environmental variables might directly affect gene expression, thus obscuring the impact of the circatidal clock on transcript levels. Intriguingly, circadian gene transcripts are usually only weakly rhythmic, or arrhythmic, during or after tidal and circadian entrainment [11,18,61–64]. Several explanations could account for these observations: (i) circadian and circatidal transcriptional rhythms are spatially restricted and only present in a small number of 'pacemaker' cells, resulting in masking of the rhythms by non-oscillating cells; (ii) the circatidal and circadian pacemakers do not rely heavily on transcriptional regulation for rhythmicity; or (iii) core components of circatidal and circadian clocks are shared but exhibit distinct oscillations in separate anatomical regions and thus mask each other. The first two possibilities seem unlikely given that both would require dramatic changes to core mechanics of the circadian clock that have otherwise been quite conserved throughout animal evolution. Also, the evidence in *P. hawaiensis* that BMAL1 serves as a component shared between the clocks seems to point towards the last possibility. There are exceptions, however. Circadian clock gene expression was monitored in oysters (*Crassostrea gigas*) from animals collected in the field or exposed to either constant conditions or light/dark cycles in the laboratory [16]. Most circadian clock genes were strongly rhythmic, but for many whether they adopted a circatidal or circadian pattern of expression was dependent on environmental conditions. Also, in arctic copepods (*Calanus finmarchicus*), circadian clock genes can show either a circadian or a circatidal pattern of expression depending on the latitude and thus the presence of permanent ice [65]. This translates in a broad change in rhythmic transcription patterns [66]. Such molecular plasticity could translate into behavioral adaptation to different rhythmic environments.

So far, circatidal transcriptomics has not uncovered obvious candidate genes for the circatidal clock (see Outstanding questions). However, it has helped to identify the physiological pathways under circatidal control. These pathways include those involved in metabolism and protein homeostasis as well as transcriptional regulation. Intriguingly, some transcripts in mouse liver and mouse cell culture also show 12-h rhythms and are enriched for genes involved in metabolism, the endoplasmic reticulum stress response, gene expression, and protein production [67,68]. These mammalian 12-h rhythms appear to be independent of BMAL1 and the mammalian circadian clock in general and could represent an adapted version of the circatidal clock in organisms living beyond the intertidal zone. X-box binding protein 1 (XBP1) is an important regulator of these rhythms, but even in its absence a significant fraction of genes remain rhythmic, with some even exhibiting more robust 12-h rhythms [68]. XBP1 thus does not seem to be a core element of the 12-h clock. Clearly, more work is needed to establish a link between mammalian 12-h rhythms and tidal rhythms in marine organisms (see Outstanding questions).

In summary, genetic studies and behavioral observations of both circadian and circatidal rhythms in a single organism support the existence of distinct circadian and circatidal clocks, which share at least one element: BMAL1 (Figure 4B). However, gene expression studies indicate that the expression of circadian genes is highly plastic in some species. In these organisms, a single clock

might be present, or perhaps the circadian clock's oscillations are driven by the circatidal clock under tidal conditions.

Circalunar clocks

Circalunar clocks are even more challenging to study than their circatidal counterparts, given their long period (29.5 days). As for circatidal rhythms, the mechanisms underlying the circalunar clock are unclear. It is possible that a dedicated 29.5-day oscillator exists, but one could also envision that a combination of circadian and circatidal oscillators is used as a coincidence detector. Circadian and circatidal clocks reach an identical phase relationship twice in a lunar month; this makes sense since the lunar month is the result of the interaction between the solar (24 h) and lunar (24.8 h) days ('beat hypothesis') [69]. This circa-semilunar interaction between the circadian and circatidal clocks could thus drive rhythms such as the aforementioned developmental/reproductive cycles of midges that peak at new and full moons. Circalunar rhythmicity could be derived from this mechanism by using moonlight cues to suppress one of the two monthly beats. Behavioral observations in *Scyphax ornatus* lend support to the beat hypothesis [70]. Changing the period of either the tidal or the solar cycle affects the period of the semilunar rhythm of locomotion. The caveat with these experiments is that behavior obviously cannot be measured under free-running conditions, and thus direct effects of environmental cycles on behavior might complicate interpretation. Zantke *et al.* presented pharmacological evidence supporting distinct circadian and circalunar clock mechanisms [14]. CKI-δ/ε inhibitors disrupted circadian locomotor rhythms but not circalunar spawning rhythms in the marine annelid *Platynereis dumerilii*, an emerging genetically tractable model for circalunar and circadian clock studies [71,72].

Trailblazing work by the Tessmar–Raible laboratory is beginning to uncover mechanisms of circalunar rhythms in *P. dumerilii*. Using TALEN scissors, a genome-engineering approach that predates CRISPR/Cas9, mutants for two photoreceptors were generated: opsin-1 and L-CRY (a light-sensitive CRY) [73]. Interestingly, the two light sensors play specific roles. Opsin-1 is necessary for proper behavioral synchronization of swarming (a locomotor activity) with the rise of the moon. L-CRY, by contrast, allows *P. dumerilii* to distinguish sunlight from moonlight. The mechanism is not yet entirely clear, but a combination of *in vivo* and *in vitro* observations provides us with important clues [73,74]. Light-sensitive CRYs undergo protein degradation when exposed to light, as a result of UV/blue-light photon absorption that triggers the reduction of their FAD chromophore and thus changes in protein conformation [75–77]. These conformational changes also allow CRY to interact with new targets (e.g., TIM in *Drosophila*). In *P. dumerilii*, L-CRY is degraded under sunlight but not moonlight. As a result, its levels and nuclear/cytoplasmic localization differ under sunlight and moonlight but are similar under moonlight and in darkness (Figure 5) [73,74]. However, it is not that L-CRY cannot detect moonlight. Spectroscopic measurement with purified L-CRY show that it does detect light at moonlight intensity [74]. However, L-CRY is activated much more slowly, and only partially, by moonlight. Since L-CRY forms dimers (at least *in vitro*), this slow and partial activation results in dimers in which only one subunit is activated, which slows the kinetics of return to the ground state, and probably also alters the conformation of the dimer. Thus, L-CRY presumably triggers different molecular responses under sunlight and moonlight, and this might be aided by the changes in L-CRY's nuclear:cytoplasmic localization ratio (Figure 5). Interestingly, in corals, CRY2 levels are correlated with moon-phase-dependent spawning [78]. In *Drosophila*, CRY improves the synchrony of circadian neurons entrained to moonlight conditions at night [73]. These observations suggest that CRY's function in moonlight detection is evolutionarily conserved.

Since *P. dumerilii*'s L-CRY and opsin-1 can detect and interpret moonlight, they would appear to be prime candidates to entrain the circalunar oscillator to moonlight. However, entrainment of the

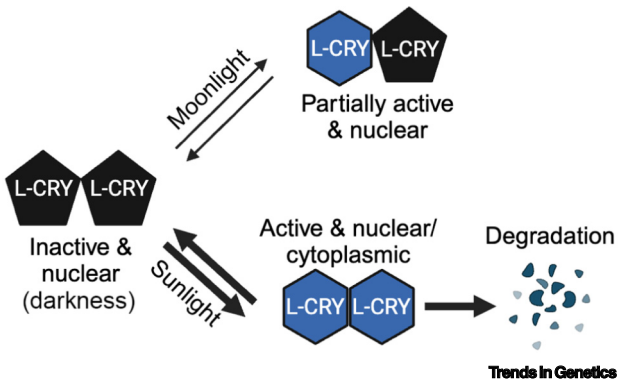

Figure 5. L-CRYPTOCHROME (L-CRY) allows *Platynereis dumerilii* to distinguish moonlight from sunlight. Moonlight slowly (small arrow) and partially activates L-CRY (blue L-CRY, light activated; black L-CRY, dark conformation). The partially activated dimer is mostly nuclear. Full, rapid (large arrow) activation by sunlight results in L-CRY being more cytoplasmic and more rapidly degraded. Differences in protein conformation and subcellular localization could trigger molecular and ultimately behavioral responses specific to moonlight and sunlight.

Outstanding questions

How much mechanistic overlap is there between the circadian and circatidal clocks?

Which genes are dedicated to the generation of circatidal rhythms?

How conserved is the molecular circatidal clock across intertidal organisms?

Are 12-h rhythms in terrestrial mammals linked to circatidal rhythms?

What is the mechanism of the circalunar clock?

How are circatidal and circalunar clock entrained to tides and the moon phase?

How do various clocks interact to produce plastic rhythms in behavior and physiology?

circalunar oscillator was not affected by loss of opsin-1 and only very mildly affected by the absence of L-CRY [74]. Rather surprisingly, under artificial lunar cycles, the spawning behavior of *l-cry*-mutant animals was more tightly synchronized than that of wild-type controls and was reminiscent of behavior under natural light. Thus, it appears that L-CRY's main role might be to recognize moonlight. It would be interesting to test mutant animals lacking both L-CRY and opsin-1 (and perhaps other opsins), since in flies circadian behavioral rhythms are entrained through both CRY and visual inputs and only the elimination of both pathways results in circadianly blind flies [79].

Concluding remarks

Historically, the study of biological clocks in marine organisms has been challenging. Marine organisms are often difficult to culture in the laboratory. Thus, there have been few marine laboratory models and until recently none was genetically tractable. Emerging model organisms such as *P. hawaiensis* and *P. dumerilii* are beginning to change the landscape. Their combination of reliable behavioral assays and genome-editing capabilities promise much progress in our understanding of marine clocks in the coming years. It is an exciting time to dive into the field of marine chronobiology.

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Declaration of interests

The authors declare no competing interests.

References

1. Hafker, N.S. *et al.* (2023) Rhythms and clocks in marine organisms. *Annu. Rev. Mar. Sci.* 15, 509–538
2. Nybakken, J.W. (2001) *Marine biology : an ecological approach* (5th edn), Benjamin Cummings
3. Neumann, D. (2014) Timing in tidal, semilunar, and lunar rhythms. In *Annual, lunar, and tidal clocks: patterns and mechanisms of nature's enigmatic rhythms* (Numata, H. and Helm, B., eds), pp. 3–24, Springer
4. Satoh, A. and Numata, H. (2014) Circatidal rhythms and their entrainment to the tidal cycle in Insects. In *Annual, lunar, and tidal clocks: patterns and mechanisms of nature's enigmatic rhythms* (Numata, H. and Helm, B., eds), pp. 25–39, Springer
5. Brown Jr., F.A. *et al.* (1954) Persistent daily and tidal rhythms of O_2-consumption in fiddler crabs. *J. Cell. Comp. Physiol.* 44, 477–505
6. Naylor, E. (1958) Tidal and diurnal rhythms of locomotor activity in *Carcinus maenas*. *J. Exp. Biol.* 35, 602–610
7. Bennett, M.F. *et al.* (1957) Persistent tidal cycles of spontaneous motor activity in the fiddler crab, *Uca pugnax*. *Biol. Bull.* 112, 267–275
8. Chabot, C.C. *et al.* (2004) Circatidal and circadian rhythms of locomotion in *Limulus polyphemus*. *Biol. Bull.* 207, 72–75
9. Zhang, L. *et al.* (2013) Dissociation of circadian and circatidal timekeeping in the marine crustacean *Eurydice pulchra*. *Curr. Biol.* 23, 1863–1873
10. Akiyama, T. (2004) Entrainment of the circatidal swimming activity rhythm in the cumacean *Dimorphostylis asiatica* (Crustacea) to 12.5-hour hydrostatic pressure cycles. *Zool. Sci.* 21, 29–38
11. Mat, A.M. *et al.* (2020) Biological rhythms in the deep-sea hydrothermal mussel *Bathymodiolus azoricus*. *Nat. Commun.* 11, 3454
12. Girard, F. *et al.* (2022) Phenology in the deep sea: seasonal and tidal feeding rhythms in a keystone octocoral. *Proc. Biol. Sci.* 289, 20221033

13. Andreatta, G. and Tessmar-Raible, K. (2020) The still dark side of the moon: molecular mechanisms of lunar-controlled rhythms and clocks. *J. Mol. Biol.* 432, 3525–3546
14. Zantke, J. *et al.* (2013) Circadian and circalunar clock interactions in a marine annelid. *Cell Rep.* 5, 99–113
15. Kwiatkowski, E.R. *et al.* (2023) Behavioral circatidal rhythms require Bmal1 in *Parhyale hawaiensis*. *Curr. Biol.* 33, 1867–1882.e5
16. Tran, D. *et al.* (2020) Bivalve mollusc circadian clock genes can run at tidal frequency. *Proc. Biol. Sci.* 287, 20192440
17. Sorek, M. *et al.* (2018) Setting the pace: host rhythmic behaviour and gene expression patterns in the facultatively symbiotic cnidarian *Aiptasia* are determined largely by *Symbiodinium*. *Microbiome* 6, 83
18. Schnytzer, Y. *et al.* (2018) Tidal and diel orchestration of behaviour and gene expression in an intertidal mollusc. *Sci. Rep.* 8, 4917
19. Holmstrom, W.F. and Morgan, E. (1983) Variation in the naturally occurring rhythm of the estuarine amphipod, *Corophium volutator* (Pallas). *J. Mar. Biol. Assoc. U. K.* 63, 833–850
20. Drolet, D. and Barbeau, M.A. (2009) Diel and semi-lunar cycles in the swimming activity of the intertidal benthic amphipod *Corophium volutator* in the upper Bay of Fundy, Canada. *J. Crustac. Biol.* 29, 51–56
21. Kaiser, T.S. *et al.* (2021) Timing strains of the marine insect *Clunio marinus* diverged and persist with gene flow. *Mol. Ecol.* 30, 1264–1280
22. Neumann, D. (1989) Circadian components of semilunar and lunar timing mechanisms. *J. Biol. Rhythm.* 4, 285–294
23. Kaiser, T.S. *et al.* (2016) The genomic basis of circadian and circalunar timing adaptations in a midge. *Nature* 540, 69–73
24. Kaiser, T.S. *et al.* (2010) Strong genetic differentiation and post-glacial origin of populations in the marine midge *Clunio marinus* (Chironomidae, Diptera). *Mol. Ecol.* 19, 2845–2857
25. Weaver, D.R. and Emery, P. (2012) Circadian timekeeping. In *Fundamental neuroscience* (3rd edn) (Squire, L.R., ed.), pp. 819–845, Academic Press
26. Hardin, P.E. (2011) Molecular genetic analysis of circadian timekeeping in *Drosophila*. *Adv. Genet.* 74, 141–173
27. Tataroglu, O. and Emery, P. (2015) The molecular ticks of the *Drosophila* circadian clock. *Curr. Opin. Insect Sci.* 7, 51–57
28. Green, C.B. (2018) Circadian posttranscriptional regulatory mechanisms in mammals. *Cold Spring Harb. Perspect. Biol.* 10, a030692
29. Allada, R. *et al.* (2001) Stopping time: the genetics of fly and mouse circadian clocks. *Annu. Rev. Neurosci.* 24, 1091–1119
30. Kotwica-Rolinska, J. *et al.* (2022) Loss of timeless underlies an evolutionary transition within the circadian clock. *Mol. Biol. Evol.* 39, msab346
31. Reitzel, A.M. *et al.* (2010) Light entrained rhythmic gene expression in the sea anemone *Nematostella vectensis*: the evolution of the animal circadian clock. *PLoS One* 5, e12805
32. Shoguchi, E. *et al.* (2013) A genome-wide survey of photoreceptor and circadian genes in the coral, *Acropora digitifera*. *Gene* 515, 426–431
33. Hunt, B.J. *et al.* (2019) *In silico* identification of a molecular circadian system with novel features in the crustacean model organism *Parhyale hawaiensis*. *Front. Physiol.* 10, 1325
34. Biscontin, A. *et al.* (2017) Functional characterization of the circadian clock in the Antarctic krill, *Euphausia superba*. *Sci. Rep.* 7, 17742
35. Yuan, Q. *et al.* (2007) Insect cryptochromes: gene duplication and loss define diverse ways to construct insect circadian clocks. *Mol. Biol. Evol.* 24, 948–955
36. Stanewsky, R. *et al.* (1998) The cryb mutation identifies cryptochrome as a circadian photoreceptor in *Drosophila*. *Cell* 95, 681–692
37. Emery, P. *et al.* (1998) CRY, a *Drosophila* clock and light-regulated cryptochrome, is a major contributor to circadian rhythm resetting and photosensitivity. *Cell* 95, 669–679
38. Emery, P. *et al.* (2000) *Drosophila* CRY is a deep brain circadian photoreceptor. *Neuron* 26, 493–504
39. Deppisch, P. *et al.* (2022) The gain and loss of cryptochrome/photolyase family members during evolution. *Genes (Basel)* 13, 1613
40. Zhang, L. *et al.* (2023) The circadian clock gene bmal1 is necessary for co-ordinated circatidal rhythms in the marine isopod *Eurydice pulchra* (Leach). *PLoS Genet.* 19, e1011011
41. Takekata, H. *et al.* (2012) RNAi of the circadian clock gene period disrupts the circadian rhythm but not the circatidal rhythm in the mangrove cricket. *Biol. Lett.* 8, 488–491
42. Takekata, H. *et al.* (2014) Silencing the circadian clock gene Clock using RNAi reveals dissociation of the circatidal clock from the circadian clock in the mangrove cricket. *J. Insect Physiol.* 68, 16–22
43. Bohn, G. (1903) Sur les mouvements oscillatoires des *Convoluta roscoffensis*. *C. R. Acad. Hebd. Seances Acad. Sci. D* 173, 576–578 (in French)
44. Reid, D.G. and Naylor, E. (1989) Are there separate circatidal and circadian clocks in the shore crab *Carcinus maenas*? *Mar. Ecol. Prog. Ser.* 52, 1–6
45. 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
46. Enright, J.T. (1976) Plasticity in an isopod's clockworks: shaking shapes form and affects phase and frequency. *J. Comp. Physiol.* 107, 13–37
47. Palmer, J.D. and Williams, B.G. (1986) Comparative studies of tidal rhythms. II. The dual clock control of the locomotor rhythms of two decapod crustaceans. *Mar. Behav. Physiol.* 12, 269–278
48. Palmer, J.D. (1997) Dueling hypotheses: circatidal versus circalunidian battle basics – second engagement. *Chronobiol. Int.* 14, 431–433
49. Satoh, A. *et al.* (2008) Circatidal activity rhythm in the mangrove cricket *Apteronemobius asahinai*. *Biol. Lett.* 4, 233–236
50. Akiyama, T. and Yoshida, M. (1990) The nocturnal emergence activity rhythm in the cumacean *Dimorphostylis asiatica* (Crustacea). *Biol. Bull.* 179, 178–182
51. Paris, M. *et al.* (2022) The crustacean model *Parhyale hawaiensis*. *Curr. Top. Dev. Biol.* 147, 199–230
52. Merlin, C. (2023) Biological timing: the crustacean *Parhyale* is rolling with the tides. *Curr. Biol.* 33, R415–R417
53. Adamovich, Y. *et al.* (2017) Rhythmic oxygen levels reset circadian clocks through HIF1α. *Cell Metab.* 25, 93–101
54. Xu, K. *et al.* (2011) The circadian clock interacts with metabolic physiology to influence reproductive fitness. *Cell Metab.* 13, 639–654
55. Karpowicz, P. *et al.* (2013) The circadian clock gates the intestinal stem cell regenerative state. *Cell Rep.* 3, 996–1004
56. Abruzzi, K.C. *et al.* (2017) RNA-seq analysis of *Drosophila* clock and non-clock neurons reveals neuron-specific cycling and novel candidate neuropeptides. *PLoS Genet.* 13, e1006613
57. Ma, D. *et al.* (2021) A transcriptomic taxonomy of *Drosophila* circadian neurons around the clock. *eLife* 10, e63056
58. Storch, K.F. *et al.* (2002) Extensive and divergent circadian gene expression in liver and heart. *Nature* 417, 78–83
59. Panda, S. *et al.* (2002) Coordinated transcription of key pathways in the mouse by the circadian clock. *Cell* 109, 307–320
60. Zhang, R. *et al.* (2014) A circadian gene expression atlas in mammals: implications for biology and medicine. *Proc. Natl. Acad. Sci. U. S. A.* 111, 16219–16224
61. Connor, K.M. and Gracey, A.Y. (2011) Circadian cycles are the dominant transcriptional rhythm in the intertidal mussel *Mytilus californianus*. *Proc. Natl. Acad. Sci. U. S. A.* 108, 16110–16115
62. Satoh, A. and Terai, Y. (2019) Circatidal gene expression in the mangrove cricket *Apteronemobius asahinai*. *Sci. Rep.* 9, 3719
63. Simpson, S.D. *et al.* (2017) The draft genome and transcriptome of the Atlantic horseshoe crab, *Limulus polyphemus*. *Int. J. Genomics* 2017, 7636513
64. Gracey, A.Y. *et al.* (2008) Rhythms of gene expression in a fluctuating intertidal environment. *Curr. Biol.* 18, 1501–1507
65. Huppe, L. *et al.* (2020) Evidence for oscillating circadian clock genes in the copepod *Calanus finmarchicus* during the summer solstice in the high Arctic. *Biol. Lett.* 16, 20200257
66. Payton, L. *et al.* (2021) Widely rhythmic transcriptome in *Calanus finmarchicus* during the high Arctic summer solstice period. *iScience* 24, 101927
67. Zhu, B. *et al.* (2017) A cell-autonomous mammalian 12 hr clock coordinates metabolic and stress rhythms. *Cell Metab.* 25, 1305–1319.e9

68. Pan, Y. *et al.* (2020) 12-h clock regulation of genetic information flow by XBP1s. *PLoS Biol.* 18, e3000580
69. Bunning, E. and Muller, D. (1961) Wie messen organismen lunare Zyklen. *Z. Naturforsch. B* 16, 391–395 (in German)
70. Cheeseman, J.F. *et al.* (2017) Circadian and circatidal clocks control the mechanism of semilunar foraging behaviour. *Sci. Rep.* 7, 3780
71. Ozpolat, B.D. *et al.* (2021) The nereid on the rise: *Platynereis* as a model system. *Evodevo* 12, 10
72. Bannister, S. *et al.* (2014) TALENs mediate efficient and heritable mutation of endogenous genes in the marine annelid *Platynereis dumerilii*. *Genetics* 197, 77–89
73. Zurl, M. *et al.* (2022) Two light sensors decode moonlight versus sunlight to adjust a plastic circadian/circalunidian clock to moon phase. *Proc. Natl. Acad. Sci. U. S. A.* 119, e2115725119
74. Poehn, B. *et al.* (2022) A cryptochrome adopts distinct moon- and sunlight states and functions as sun- versus moonlight interpreter in monthly oscillator entrainment. *Nat. Commun.* 13, 5220
75. Lin, F.J. *et al.* (2001) Photic signaling by cryptochrome in the *Drosophila* circadian system. *Mol. Cell. Biol.* 21, 7287–7294
76. Busza, A. *et al.* (2004) Roles of the two *Drosophila* CRYPTOCHROME structural domains in circadian photoreception. *Science* 304, 1503–1506
77. Ozturk, N. *et al.* (2011) Reaction mechanism of *Drosophila* cryptochrome. *Proc. Natl. Acad. Sci. U. S. A.* 108, 516–521
78. Levy, O. *et al.* (2007) Light-responsive cryptochromes from a simple multicellular animal, the coral *Acropora millepora*. *Science* 318, 467–470
79. Helfrich-Forster, C. *et al.* (2001) The circadian clock of fruit flies is blind after elimination of all known photoreceptors. *Neuron* 30, 249–261