Title: Epigenetic regulation of biological rhythms: an evolutionary ancient molecular timer.

Author: Tyler J Stevenson

Affiliation: Institute of Biological and Environmental Sciences, University of Aberdeen

Author contact:
Institute of Biological and Environmental Sciences
University of Aberdeen
Aberdeen, AB24 2TZ
United Kingdom

Disclosure statement: The author has nothing to disclose

Figures: 3

Key words: methylation, acetylation, circadian, infradian

Abstract

Biological rhythms are pervasive in nature, yet our understanding of the molecular mechanisms that govern timing is far from complete. The rapidly emerging research focus on epigenetic plasticity has revealed a system that is highly dynamic and reversible. In this Opinion, an epigenetic clock model is proposed that outlines how molecular modifications, such as DNA methylation, are an integral component for endogenous biological rhythms. The model provides a novel framework for the environmental and hormonal regulation of endogenous epigenetic oscillations. The hypothesis proposed is that the epigenetic clock model serves to maintain the period of molecular rhythms via control over the phase of gene transcription and this timing mechanism resides in all cells: from unicellular to complex organisms.

Introduction

The daily and yearly revolutions of Earth have provided a constantly changing environment which has driven the evolution of biological rhythms. The ability to adapt to future, predictable climatic conditions is an ancient adaptation; therefore it should not be surprising to observe biological rhythms at genomic, physiological and behavioural levels across taxa [1]. The vast majority of biological rhythms are governed by an endogenous mechanism that provides cell-autonomous oscillations in molecular and cellular signaling pathways. The most abundant evidence to support endogenous clock mechanisms includes a number of circadian clock genes (e.g. *bmal1*) that provides an internal daily rhythms [2,3]. These circadian clock genes form a self-sustained 24 hr transcriptional-translational feedback loop and is present in plants, invertebrate and vertebrate species [2,3]. Recent developments in the field of **epigenetics** (Glossary Box) have revealed dynamic molecular mechanisms with implications for effects on gene transcription across multiple time scales. Indeed the major and leading epigenetic modifications are **transgenerational epigenetic** [4] and **developmental epigenetics** [5]**.** The observation of oscillations in epigenetic enzymes indicates a mechanism that may be an integral component of the biological representation of recurrent (cyclical) time.

In this Opinion, the proposition that epigenetic modifications are an evolutionary ancient and essential component of the genomic regulation of biological rhythms is proposed. The central premise of the epigenetic clock model is that the timing, or **rhythmic epigenetic** modifications impacts normal and pathological genomic states across the lifespan and subsequent generations. The evidence is derived from data that support a link between circadian clock genes and hormonal pathways, and the cell- and tissue specific expression of enzymes involved in DNA methylation and histone acetylation. First, the various rhythmic periods over which biological rhythms have been observed is reviewed, followed by an overview of the epigenetic modifications that have mapped onto such timescales. Next, the paper will present a model that describes how epigenetic modifications may constitute a key

55 molecular signal that integrates endogenous and exogenous cues to maintain the period of biological
56 clocks.
57
58 Variation in biological rhythms
59 There are multiple time scales studied in nature and are generally divided into **ultradian**,
60 **circadian**, and **infradian** cycles. Across all scales, the environment entrains an internal mechanism
61 that drives biological oscillations, such as the circadian clock [6,7]. By far, circadian rhythms are the
62 most commonly studied biological oscillation. The identification of core circadian clock genes has
63 provided a major advancement for the development of gene-rhythm analyses for the internal
64 representation of daily time (e.g. *clock* [8]; *bmal1*[9,10]). One conjecture that has received the largest
65 focus is that expression of circadian clock genes (e.g. BMAL1) is a critical component for genomic
66 oscillatory mechanisms for other timescales such as **circannual** rhythms [11,12]. Indeed there is
67 support that circadian clock genes exhibit variation in expression under different timescales and
68 latitudinal clines [13]; however, a functional link has remained elusive. Alternatively, there is evidence
69 that non-circadian rhythms (e.g. circatidal [14], circalunar [15]) can be dissociated from circadian
70 clock gene signalling. Studies employing molecular, lesion and behavioral analyses have implicated an
71 endogenous molecular component involved in the regulation over short- and long-term time scales. For
72 example, circannual rhythms can be maintained in the absence of circadian clock gene oscillations in
73 reindeer (*Rangifer tarandus*) [16]. Lesions of the central **pacemaker** for circadian rhythms, the
74 suprachiasmatic nucleus (SCN) did not abolish circannual rhythms of body mass in ground squirrels
75 (*Spermophilus lateralis*) [17]. Circalunar rhythms in the regulation of worm (*Platynereis dumerilii*)
76 maturation remain intact despite disruption of circadian clock gene expression induced by the
77 inhibitory effects of casein kinase 1 δ/ε [15]. Moreover, circatidal behavior of locomotor activity in
78 *Eurydice pulchra* can be dissociated from circadian genes and daily activity patterns [14]. Altogether,

these data indicate that rhythmic circadian clock gene expression is not necessary for ultradian or infradian endogenous timing mechanisms and suggest an alternative mechanism could provide vital molecular timing across ultradian, infradian and circadian scales.

Core molecular mechanisms that control epigenetic modifications

Epigenetic modifications can change the probability of gene transcription across the genome and the inhibition or permissiveness of timing gene transcription occurs at a developmental, cell- and tissue- specific level [18]. Circadian clock gene control of daily rhythms involves well-described transcriptional-translational feedback loops that are present in algae, fungi, plants and animals [19-21]. One fundamental aspect of these loops is the control of gene transcription. Epigenetic modifications such as **DNA methylation** and **histone acetylation** are emerging as robust mechanisms for regulating the probability of gene transcription in these systems [22,23]. Because epigenetic modifications are present across the diverse life forms, these mechanisms may represent an evolutionary ancient timing system for the control of gene transcription.

DNA methylation and histone acetylation are the more commonly studied of the epigenetic modifications. DNA methyltransferases (*dnmt*) are enzymes that catalyze the transfer of methyl (CH3) onto nucleotides with a high preference for cytosine residues that are 5' to guanine [22]. The active removal of methylation involves another class of enzymes referred to as Ten-eleven translocation (*tet*) that oxidize the methyl groups of methylcytosine [24,25]. The predominant research has focussed on TET function in germ lines and during early development, yet there is evidence for active demethylation in adult brains and proposed to maintain CpG islands in an unmethylated state [26]. TET enzymes are also directly and indirectly involved in the regulation of other processes (e.g. DNA repair and genomic instability [27]). A similar complementary system occurs for histone acetylation: two classes of enzymes called histone acetyltransferases and histone deacetyltransferase are involved

in the addition and removal of acetyl-groups from histones respectively [23]. It is generally assumed that DNA methylation and histone deacetylation occur in tandem and significantly inhibit gene transcriptions [28]. There is growing evidence that both DNA methylation and histone acetylation are dynamic processes and exhibit predictable patterns in expression over multiple time scales. If biological rhythms in epigenetic modifications are pervasive across time scales, then it is likely that DNA methylation, for example, is a conserved molecular regulator of gene transcription and is an integral component of the core endogenous clock mechanism that governs biological timing across multiple timescales.

Across all known living organisms, nucleotides are the fundamental structure present in cells and are the building blocks that provide information necessary to exhibit rhythmic gene transcription. Here I offer the conjecture that a conserved or common mechanism for rhythmic control of gene transcription may include a structural modification to the template, such as DNA methylation, or chromatin remodelling. An overabundance of data in molecular biology, separate from the domain of chronobiology, indicate that DNA methylation is an evolutionary-conserved molecular system for the regulation of gene transcription [29,30]. The accumulation of DNA methylation in key genomic regions (i.e. promoter regions) provides a gradual silencing of target genes, and this mechanism may permit an oscillatory pattern in RNA expression. Given that DNA methylation, in particular, has been reported across the vast majority of living organisms [22,24,29,31] (Fig 1a), structural changes including epigenetic modifications could be an evolutionary conserved timing mechanism (Fig 1b). Over the course of evolutionary time, cells may have adapted to new cellular environments and developed complex transcriptional-translational loops, perhaps similar to those observed in extant species. Indeed there is high sequence and functional similarities of core circadian clock genes between Drosophila and mammalian period (*per*) and *bmal1*, suggesting these genes are components of a conserved assembly of genetic circadian clock genes [19] (Fig 1c,d). Current evidence suggests that

DNA methylation is implicated in the control of *bmal1* gene transcription [32]; there is a high density of BMAL1 binding sites (i.e. E-BOX) in one *dnmt3a* promoter [33] and crucially, BMAL1 exhibits a circadian rhythm in binding *dnmt3a* genomic regions that preceded *dnmt3a* expression [34] (Fig 2a). These data indicate that a major circadian clock gene (BMAL1) directly regulates at least one epigenetic enzyme and conversely, there is evidence that DNA methylation impacts BMAL1 expression. Therefore, a viable (and testable) hypothesis is that DNMT3a and BMAL1 signalling participates as key anchors in a daily negative feedback loop (Fig 1c,d).

Evidence for rhythms in epigenetic modifications

High-throughput analyses, such as microarray studies have revealed robust changes in multiple epigenetic enzymes. For example, the annual photoperiodic response in Japanese quail (*Coturnix japonica* [35]) and white-crown sparrows (*Zonotrichia leucophrys* [36]) was observed to include large change in enzymes involved in DNA methylation in the hypothalamus. Furthermore, a survey of the CircaDB, an online database of circadian transcriptional profiles in mammalian tissue revealed that several epigenetic enzymes (i.e. *dnmt3a, dnmt3b, hdac4 and tet2*) display tissue- and rhythmic expression patterns. The most abundant evidence to support the conjecture that epigenetic modifications are a key mechanism that controls oscillations in gene transcription are derived from circadian changes in locomotor activity [37,38] and energy balance [39-42], and from studies of circannual timing in plant vernalization [43], reproduction [44-27] and immune function [38]. One DNA methyltransferase, *dnmt3a*, has been found to exhibit marked variation in expression that conforms to multiple biological rhythms.

In hamsters, clear circadian rhythms in *bmal1* and *dnmt3a* expression occur in **neuroendocrine** substrates (Fig2a) [47]. Similar to other rodent species, *bmal1* expression increases shortly before lights-on and peaks shortly before lights-off (Fig 2b). *dnmt3a* expression is in anti-phase with *bmal1* as

the levels are relatively low during the light phase and peak during the dark phase. Overall, the circadian waveform, high-density binding sites for *bmal1* in the *dnmt3a* promoter, and evidence for daily variation in *bmal1* promoter methylation, suggest a potential negative-feedback circuit for *bmal1* driven expression of *dnmt3a* (and vice versa). In addition to circadian rhythms, hamsters exhibit marked seasonal rhythms in reproduction and energy balance. Prior work had indicated that the hypothalamus of hamsters has significantly greater levels of global DNA methylation in long summer days (LD) compared to short winter days (SD) in the laboratory [46]. These observations have since been extended to include reproductive tissues (e.g. testes) [44]. The striking reduction in testes mass after adaptation to SD is accompanied by a significant increase in global DNA methylation (Fig 2c), which may be due to increased *dnmt3a* expression (Fig 2d). It is important to emphasize that annual rhythms in DNA methylation are not limited to mammalian species, but have also been identified in plants (*Arabidopsis thaliana* [43]) and insects (*Nasonia vitripennis* [49]). One hypothesis is that circannual variation in DNA methyltransferase expression is an integral component of the seasonal regulation of gene transcription in multiple tissues [50]. Experimental data to confirm the functional role of DNA methylation for timing biological rhythms is limited. 3-aminobenzamide (3AB) is an inhibitor of poly(ADP ribosyl)ation and promotes methyl binding to DNA leading to increased global methylation. Siberian hamsters that received daily subcutaneous injections of 3AB significantly delayed annual reproductive involution responses [46]. These data suggest that hypermethylation at critical pointss in the annual cycle can delay the phase of reproductive responses. Overall, these data indicate that DNA methylation in particular, oscillates over circadian and circannual time scales and serves to regulate the timing of physiological processes critical to seasonal environmental adaptation (e.g. reproduction) [51].

There is growing evidence that the time of food intake has a remarkable level of bi-directional interactions between the circadian clock and energy balance [52]. The vast majority of research has

examined the contribution of chromatin remodelling via the activity of sirtuin proteins (SIRT1) within well-defined neuroendocrine circuits that govern energy balance [52]. Increased SIRT1 activity leads to histone deacetylation and can directly interact with circadian clock genes. In particular, SIRT1 removes histone acetyl groups via a nicotinamide adenine dinucleotide (NAD+)-dependent manner (reviewed by [53]). Crucially, SIRT1 has been shown to physically interact with CLOCK; and regulates clock controlled genes in a histone deacetylation dependent manner [41,42]. Subsequent research has shown that SIRT1 expression specifically within Sf1 neurons in the ventromedial nucleus of the hypothalalmus (VMN) are a critical node for the coordination of circadian behaviour and daily energetic state.

Framework for an Epigenetic Clock Model

Here an Epigenetic Clock Model is proposed, outlined in Figure 3. Given the abundance of information on the circadian regulation of physiology and behaviour, the model will first outline how an epigenetic clock functions within this transcriptional-translational feedback network. Then, the role of epigenetic modifications for the regulation of infradian (e.g. circannual) rhythms will be described. The core hypothesis is that epigenetic modifications controls the phase in gene transcription and in turn maintains the period of the biological rhythm. Due to the relatively abundant information on DNA methylation, the model will reflect this bias. Indeed there is growing evidence that the location of DNA methylation, such as promoters, gene bodies, regulatory elements and repeat sequences have a range of different functions [54]. The model presented herein primarily focuses on the inhibitory role of DNA methylation on gene transcription.

In mammals, daily rhythms are coordinated by intrinsic oscillations within the central pacemaker, the suprachiasmatic nuclei (SCN) that is synchronized with exogenous and endogenous cues (e.g. light, energy balance). Non-visual image light information is transmitted via melanopsin

expressing retinal-ganglion cells to the SCN [55]. Daily **photoperiodic** information entrains circadian clock genes that form a transcriptional-translational feedback loop that approximates a 24hr period. The circadian clock gene *bmal1* expression in the SCN as well as the vast majority of cells within the central and peripheral clocks is a key component for the genetic regulation of circadian clock-controlled genes [19,21,56]. The presence of E-box elements in the promoter region for *dnmt3a* provides the necessary link between the binding of a core circadian clock gene (i.e. BMAL1) and an effector of epigenetic modifications (Fig 2b). In combination with the data reported by Azzi and colleagues [37,38], it is likely that *dnmt3a* is a target for BMAL1 [34] and leads to the epigenetic control of circadian rhythms in activity. One remarkable observation has been the relatively small overlap in the phase of circadian clock genes between cells indicating a high level of tissue- and cell-autonomous genomic regulation of daily cycling of gene expression [56]. The Epigenetic clock model indicates that BMAL1 driven changes in *dnmt3a* may be a key output signal to provide tissue- and cell- specificity in circadian control of gene transcription. The circadian rhythms in *dnmt3a*, for example, may lead to increased methylation of target promoter regions [37]. The increased DNA methylation likely directs cell-specific gene transcription and thus, governs the daily epigenomic landscape. The removal of methyl groups, in this case, occurs over a period that approximates 24hrs. One exciting corollary of this model is that, given that DNA methylation is an evolutionary ancient mechanism, circadian rhythms in DNA methylation (or other epigenetic modifications) *per se* may predate the presently established collection of gene transcripts that constitute transcriptional-translational feedback loop as we know it today.

One of the biggest challenges in the field of chronobiology is uncovering the molecular and cellular control of non-circadian rhythms, such as infradian rhythms. The two predominant infradian rhythms in which there is evidence for epigenetic plasticity include circannual and **circaquadridian**. Annual rhythms have already been discussed and there is support derived from strong associations

between photoperiodic regulation seasonal rhythms and DNA methylation in plants [43], invertebrate [49] and vertebrate species [45,46]. It remains possible that circannual rhythms generated and maintained by epigenetic modifications could be driven, at least in part, by annual changes in circadian clock genes. However, the evidence that SCN-lesions in ground squirrels [17] and molecular oscillatory patterns in gene expression in reindeer [16] together indicate that circannual rhythms in epigenetic modifications may be independent of circadian timing mechanisms. Therefore, the long-term internal representation of circannual time could be driven by tissue and cell-specific epigenetic modifications. Here, the annual DNA methylation of key genomic regions would be proposed to approximate 365 days and coordinate the seasonal timing of gene transcription required for the expression of circannual rhythms in physiology and behaviour. Epigenetic modifications are hypothesized to be integral components of the endogenous long-term timer and entrained by seasonal environmental cues (e.g. photoperiod) [50].

Mice and rats exhibit a clear circaquadridian estrous cycle that has been extremely valuable for medical research, particularly reproductive physiology and fertility. In particular, recent advances in reproductive physiology had identified a discrete neuropeptidergic population referred to as kisspeptin (KiSS1) that provides negative and positive feedback during the estrous cycle [57,58]. Current evidence indicates the estrogen-dependent positive feedback leads to chromatin remodelling that enhances KiSS1 signalling in the Anteroventro-pericentricular nucleus (AvPv) [57]. These data demonstrate that cyclical patterns in histone acetylation over the rodent estrous cycle are critical for the timing of ovulation. Recent evidence collected in the hamster uterus has indicated that *dnmt3a* also varies across the estrous cycle with peak levels during proestrous that subsequently decrease during the estrous phase (Fig 2e) [44]. The reduction in *dnmt3a* levels are driven by increased estrogen secretion from the ovary [44]. Given the observation for cyclical variation in *dnmt3a* expression in peripheral

reproductive tissues, such as the uterus, it is likely that variation in DNA methylation and histone acetylation modifications are widespread across the female cycle and occur in a tissue-specific manner.

To date, there are no data available to support the potential for cyclical epigenetic modifications over ultradian timescales. The most fruitful approach to identify and examine the role of DNA methylation, in particular, for the regulation of short term oscillations (i.e. ~6hr) are well-known pulsatile nature of many secretagogues. For example, genomic plasticity includes discrete neuropeptide cell cultures (e.g. GT1 gonadotropin-releasing hormone cell lines). Once activated, DNMT enzymes can methylate DNA within 20 minutes and effectively silence gene transcription [59,60]; therefore, such a mechanism could occur within the high-frequency timescales necessary to participate in the generation of ultradian rhythms.

Concluding Remarks and Future Perspectives

In conclusion, this paper has proposed that epigenetic modifications are central to the period of multiple biological rhythms, over short- and long-term timescales. How epigenetic modifications conform to well-established molecular timing systems is poorly described (Outstanding Questions Box), thus, there is ample and exciting prospects for major advances in our understanding of short- and long-term control over the timing of gene transcription. Specifically, it is likely that circadian disruptions have a detrimental effect on epigenetic signalling cascades and likely contribute to increased acute and chronic illnesses and diseases.

The level of epigenetic oscillations in the molecular and cellular control of cell/tissue function is quite complex. One limitation is uncovering how the respective modifications, such as DNA methylation and histone acetylation, contribute to timing across vertebrate and invertebrate species (Fig. 1a). DNA methylation in particular has been identified in the vast majority of organisms, however, in some, such as yeast, there appears to be a complete absence of DNA methylation [31]. The loss of DNA methylation is particularly intriguing and indicates that, in yeast, oscillations in the

epigenomic landscape have a limited role in the maintenance of biological rhythms. Alternatively, yeast show global and specific chromatin remodelling via histone acetylation and deacetylation providing the potential for the endogenous cycling of chromatin [61]. Other epigenetic modifications could also exhibit rhythmic effects at transcriptome (e.g. RNA methylation; [62]) and proteome (e.g. protein methylation) levels. Recent work has indicated that oscillatory cytosolic mechanisms can be integrated with biological clocks (i.e., circadian) independent of transcriptional-translational feedback loops [63]. One tantalizing mechanism consists of oscillations in epigenetic modifications in cytosolic signaling. Protein methylation is a relatively understudied mechanism; current evidence suggests protein methylation regulates a wide-range of processes including gene transcription, chromatin remodelling and signal transduction [64]. Altogether, rhythmic epigenetic mechanisms may regulate short- and long-term molecular and cellular signaling pathways at multiple levels.

Across all the data presented, the *de novo* DNA methylation enzyme, *dnmt3a* is the central focus due to the robust evidence in support of oscillations in gene expression. It is important to highlight that other epigenetic enzymes, involved in other modifications, such as histone acetylation are likely involved. The model described herein provides a novel epigenetic framework for a series of research questions that stand to provide significant advancements for our understanding of the molecular control of gene expression [65-67]. Given the recent emphasis of circadian and circannual rhythms for the maintenance of behaviour, cognition and mental well-being and the consequences of daily and seasonal disruption for increased pathology [68,69], a greater understanding of cyclical patterns in epigenetic modifications is warranted.

Acknowledgements

The author thanks Barbara Helm, John O'Neill and Brian Prendergast for constructive comments on a previous version of the manuscript.

References

1. Hut, R.A., and Beersma, D.G. (2011) Evolution of time-keeping mechanisms: early emergence and adaptation to photoperiod. Philos. Trans. R. Soc. Lond. B. Biol. Sci. 366, 2141-2154.

2. Bell-Pedersen, D. et al. (2005) Circadian rhythms from multiple oscillators: lessons from diverse organisms. Nat. Rev Genetics 6, 544-556.

3. Hastings, M.H. Reddy, A.B. Maywood, E.S. (2003) A clockwork web: circadian timing in brain and periphery, in health and disease. Nat. Rev. Neurosci. 4, 649-661.

4. Daxinger, L. Whitelaw, E. (2012) Understanding transgenerational epigenetic inheritance via the gametes in mammals. Nat Rev Genet. 13, 153-162.

5. Feng, S. Jacobsen, S.E. Reik, W. (2010) Epigenetic reprogramming in plant and animal development. Science 330, 622-627.

6. Roenneberg, T. et al. (2003) The art of entrainment. J. Biol. Rhythm 18, 183-194.

7. Zordan, M.A. et al. (2001) Photic entrainment of the circadian clock: from Drosophila to mammals. Semin. Cell. Dev. Biol. 12, 317-328.

8. Vitaterna, M.H. et al. (1994) Mutagenesis and mapping of a mouse gene, Clock, essential for circadian behavior. Science 264, 719-725.

9. Hogenesch, J.B. et al. (1997) Characterization of a subset of the basic-helix-loop-helix-PAS superfamily that interacts with components of the dioxin signaling pathway. J Biol Chem. 272, 8581-8593.

10. Ikeda, M. and Nomura, M. (1997) cDNA cloning and tissue-specific expression of a novel basic helix-loop-helix-PAS protein (*bmal1*) and identification of alternatively spliced variants with alternative translation initiation site usage. Biochem. Biophys. Res. Commun. 233, 258-264.

11. Meuti, M.E. and Denlinger, D.L. (2013) Evolutionary links between circadian clocks and photoperiodic diapauses in insects. Integr. Comp. Biol. 53, 131-143.

12. Pegoraro, M. et al. (2014) Role for circadian clock genes in seasonal timing: testing the Bunning hypothesis. PLoS Genet. 10, e1004603.

13. Yammouni, R. Bozzano, A. Douglas, R.H. (2011) A latitudinal cline in the efficacy of endogenous signals: evidence derived from retinal cone contraction in fish. J. Exp. Biol. 214, 501-508.

14. Zhang, L. et al. (2013) Dissociation of circadian and circatidal timekeeping in the marine crustacean *Eurydice pulchra*. Curr. Biol. 23, 1863-1873.

15. Zantke, J. et al. (2013) Circadian and circalunar clock interactions in a marine annelid. Cell. Rep. 5, 99-113.

16. Lu, W. et al. (2010) A circadian clock is not required in an arctic mammal. Curr. Biol. 20, 533-537.

17. Dark, J. et al. (1985) Persistence of circannual rhythms in ground squirrels with lesions of the suprachiasmatic nuclei. Brain Res. 332, 201-207.

18. Li, E. (2002) Chromatin modification and epigenetic reprogramming in mammalian development. Nat. Rev. Gen. 3, 662-673.

19. Lee, K. et al. (2000) Interconnected feedback loops in the Neurospora circadian system. Science 289, 107-110.

20. Roenneberg, T. and Merrow, M. (2002) Cell Tissue Res. “What watch? … such much!” Complexity and evolution of circadian clocks. Cell Tissue Res 309, 3-9.

21. Mohawk, J.A. et al. (2012) Central and peripheral circadian clocks in mammals. Ann. Rev. Neurosci. 35, 445-462.

22. Schubeler, D. (2015) Function and information content of DNA methylation. Nature 517, 321-326.

23. Struhl, K. (1998) Histone acetylation and transcriptional regulatory mechanisms. Gene. Develop. 12, 599-606.

24. Wu, H. and Zhang, Y. (2014) Reversing DNA methylation: mechanisms, genomics and biological functions. Cell 156, 45-68.

25. Carey, N. Marques, C.J. Reik, W. (2011) DNA demethylases: a new epigenetic frontier in drug discovery. Drug Disc Today 16, 683-690.

26. Williams, K. et al. (2012) DNA methylation: TET proteins-guardians of CpG islands? EMBO Rep. 13, 28-35.

27. Jeltsch, A. Jurkowska, R.Z. (2014) New concepts in DNA methylation. Trends Biochem. Sci 39, 310-318.

28. Cameron, E.E. et al. (1999) Synergy of demethylation and histone deacetylase inhibition in the re-expression of genes silenced in cancer. Nat. Genet. 21, 103-107.

29. Steele, R.E. and Rae, P.M.M. (1980) Ordered distribution of modified bases in the DNA of a dinoflagellate Nucleic. Acids Res. 8, 4709-4726.

30. Helm, B. and Stevenson, T.J. (2014) Circannual Rhythms: History, Present Challenges, Future Directions. In: *Annual, Lunar and Tidal Clocks: Patterns and Mechanisms of Nature's Enigmatic Rhythms,* Eds Helm, B., & Numata, H. Springer, Japan. Pg. 203-225.

31. Capuano, F. et al. (2014) Cytosine DNA methylation is found in *Drosophila melanogaster* but absent in *Saccaromyces cerivisiae, Schizosaccharomyces pombe,* and other Yeast species. Anal. Chem. 86, 3697-3702.

32. Satou, R. et al. (2013) DNA methylation of the *bmal1* promoter. Biochem. Biophys. Res. Commun. 440, 449-453.

33. Yanagisawa, Y. et al. (2002) The human DNA methyltransferases DNMT3a and DNMT3b have two types of promoters with different CpG contents. Biochimica et Biophysica Acta. 1577, 457-465.

34. Koike, N. Et al. (2012) Transcriptional architecture and chromatin landscape of the core circadian clock in mammals. Science 2012; 338:349-354.

35. Nakao, N. et al. (2008) Thyrotrophin in the pars tuberalis triggers photoperiodic response. Nature 452, 317-322.

36. Mukai, M. et al. (2009) Seasonal differences of gene expression profiles in song sparrow (*Melospiza melodia*) hypothalamus in relation to territorial aggression. PLoS One 4, e8182.

37. Azzi, A. et al. (2014) Circadian behavior is light-reprogrammed by plastic DNA methylation. Nat Neurosci. 17, 377-382.

38. Azzi, A. et al. (2017) Network dynamics mediate circadian clock plasticity. Neuron 93, 441-450.

39. Aguilar-Arnal, L. and Sassone-Corsi, P. (2015) Chromatin landscape and circadian dynamics: spatial and temporal organization of clock transcription. Proc. Natl Acad. Sci 112, 6863-6870.

40. Masri, S. and Sassone-Corsi, P. (2013) The circadian clock: a framework linking metabolism, epigenetics and neuronal function. Nat. Rev. Neurosci. 14, 69-75.

41. Nakahata, Y. Et al. (2008) The NAD+-dependent deacetylase SIRT1 modulates CLOCK-mediated chromatin remodelling and circadian control. Cell 134, 329-340.

42. Orozco-Solis R, Ramadori G, Coppari R, Sassone-Corsi P. (2015) SIRT1 relays nutritional inputs to the circadian clock through the Sf1 neurons of the ventromedial hypothalamus. Endocrinol 156, 2174-2184.

43. Finnegan, E.J. et al. (1998) DNA methylation and the promotion of flowering by vernalization. Proc Natl Acad Sci USA 95, 5824-5829.

44. Lynch, E.W.J. et al. (2016) Cyclical DNA methyltransferase 3a expression is a seasonal and oestrous timer in reproductive tissues. Endocrinol. 157, 2469-2478.

45. Alvarado, S. et al. (2015) Dynamic changes in global and gene-specific DNA methylation during hibernation in adult thirteen-lined ground squirrels, *Ictidomys tridecemlineatus*. J Exp Biol 218, 1787-1795.

46. Stevenson, T.J. and Prendergast, B.J. (2013) Reversible DNA methylation regulates seasonal photoperiodic time measurement. Proc. Natl. Acad. Sci. 110, 16651-16656.

47. Stevenson, T.J. (2017) Circannual and circadian rhythms of DNA methyltransferase and histone deacetylase enzymes in the Siberian hamsters (*Phodopus sungorus*) hypothalamus. Gen. Comp. Endocrinol. 243, 130-137.

48. Stevenson, T.J. et al. (2014) Cell-autonomous iodothyronine deiodinase expression mediates seasonal plasticity in immune function. Brain Behav. Immuno. 36, 61-70.

49. Pegoraro, M. et al. (2016) DNA methylation changes induced by long and short photoperiodis in Nasonia. Genome Res 26, 203-210.

50. Stevenson, T.J. and Lincoln, G.A. (2017) Epigenetic Mechanisms Regulating Circannual Rhythms. In *Biological Timekeeping: Clocks, Rhythms and Behaviour* (Kumar V., ed.) Springer, India.

51. Stevenson, T.J. (2017) Environmental and hormonal regulation of epigenetic enzymes in the hypothalamus. J. Neuroendocrinol. 29, 1-9.

52. Asher, G. Sassone-Corsi, P. (2015) Time for food: the intimate interplay between nutrition, metabolism and the circadian clock. Cell 161, 84-92.

53. Masri S, Sassone-Corsi P. (2013) The circadian clock: a framework linking metabolism, epigenetics and neuronal function. Nat Rev Neurosci 14, 69-75.

54. Jones, P.A. (2012) Functions of DNA methylation: islands, start sites, gene bodies and beyond. Nat Rev. Genet. 13:484-492.

55. Peirson, S. and Foster, R.G. (2006) Melanopsin: another way of signaling light. Neuron 49, 331-339.

56. Panda, S. et al. (2002) Coordinated transcription of key pathways in the mouse by the circadian clock. Cell 109, 307-320.

411 57. Tomikawa, J. et al. (2012) Epigenetic regulation of Kiss1 gene expression mediating estrogen-
412 positive feedback action in the mouse brain. Proc. Natl. Acad. Sci. 109, 7609-7610.
413 58. Kauffman, A.S. et al. (2007) Emerging ideas about Kisspeptin-GPR54 signaling in the
414 neuroendocrine regulation of reproduction. Trends Neurosci. 30, 504-511.
415 59. Aoki, A. et al. (2001) Enzymatic properties of de novo type mouse DNA (cytosine-5)
416 methyltransferases. Nucl Acids Res. 29,3506-3512.
417 60. Hermann, A. et al. (2004) The Dnmt1 DNA-(cytosine-C5)-methyltransferase methylates DNA
418 processively with high preference for hemimethylated target sites. J. Biol. Chem. 279, 48350-
419 48359.
420 61. Vogelauer M. Wu, J. Suka, N. Grunstein, M. (2000) Global histone acetylation and deacetylation
421 in yeast. Nature 408, 495-498.
422 62. Fustin, J.M. et al. (2013) RNA-methylation-dependent RNA processing controls the speed of the
423 circadian clock. Cell 155, 793-806.
424 63. Hastings, M.H. et al. (2008) Cellular circadian pacemaking and the role of cytosolic rhythms. Curr
425 Biol. 18, R805-R815.
426 64. Biggar, K.K. and Li, S.C. (2015) Non-histone protein methylation as a regulator of cellular
427 signaling and function. Nature Rev Mol Cell Biol 16, 5-17.
428 65. Auclair, G. et al. (2014) Ontogeny of CpG island methylation and specificity of DNMT3
429 methyltransferases during embryonic development in the mouse. Genome Biol. 15, 545.
430 66. Shahbazian, M.D. and Grunstein, M. (2007) Functions of site-specific histone acetylation and
431 deacetylation. Ann. Rev. Biochem. 76, 75-100.
432 67. McDonald, J.I. et al. (2016) Reprogrammable CRISPR/CAS9-based system for inducing site-
433 specific DNA methylation. Biol. Open 10.1242/bio.019067.

68. Wulff, K. et al. (2010) Sleep and circadian rhythm disruption in psychiatric and neurodegenerative disease. Nat. Rev. Neurosci. 11, 589-599.

69. Stevenson, T.J. et al. (2015) Disrupted seasonal biology impacts health, food security and ecosystems Proc. Biol. Sci B. 282, 1-10.

Figure Legends

Figure 1 Phylogenetic occurrence of biological rhythms in DNA methylation. A) Phylogenetic tree indicating the presence (solid line) and absence (dashed line) of DNA methylation. B) In a hypothetical evolutionary ancient cell, a negative feedback system could control biological rhythms in gene transcription. The detection of a periodical environmental stimulus, indicated by sinewave, is hypothesized to trigger the expression of an epigenetic modifier (EM; e.g. DNMT, HDAC). Increased EM expression then initiates a biochemical modification surrounding the DNA template; and governs the probability of gene transcription at targeted loci. In a simplified system, the EM provides negative feedback leading to the reduction in *em* transcription. Circadian rhythms in DNA methyltransferase (DNMT) expression provide daily epigenetic control over the timing of gene transcription. C) and D) are hypothetical schematic diagrams of epigenetic modifications via DNMT in Drosophila melanogaster and Homo sapiens, respectively. Cycle (CYC) and Aryl hydrocarbon receptor nuclear translocator-like BMAL1) proteins are two well described components of the molecular circadian clock. In these models, daily timing in gene transcription is provided by an oscillation in clock gene stimulation of DNMT, which in turn, increases DNA methylation in the promoter regions providing negative feedback leading to an inhibition of CYC/BMAL1 expression. The precise relationship between epigenetic enzymes (e.g. DNMT) and well-established circadian positive- and negative-feedback loops is yet to be determined.

Figure 2 Current evidence for biological rhythms in DNA methylation and DNA methyltransferase enzyme expression. Siberian hamsters exhibit robust ultradian, circadian and infradian rhythms. (A) *dnmt3a* expression exhibits a circadian waveform that is in anti-phase with *bmal1* expression. The white-black bar across the top indicates lights on - lights off respectively. (B) Consite analyses of one *dnmt3a* promoter region located between exon 1b and 1c indicates a series of BMAL1 binding sites (i.e. E-Box) motifs. An circannual rhythm in testicular function (i.e. mass) is one control mechanism involved in the timing of seasonal reproduction. (C) In summer-long day (LD) phenotypes, hamsters are reproductively competent with large testes; upon exposure to winter-short days (SD) testes regress and reproduction is terminated. The global level of DNA methylation significantly increases in SD-regressed testes and is associated with the inhibition of spermatogenesis. (D) The seasonal rhythm in testicular DNA methylation is proposed to be under the control of DNMT3a as SD testes exhibit significantly greater levels of expression. Finally (E) *dnmt3a* expression also exhibits short-term estrous rhythms (i.e. circaquadridian), in particular, significant reductions occur in the uterus endometrium during the estrous cycle.

Figure 3 Key Figure: schematic diagram of the Epigenetic Clock model for the regulation of biological rhythms. Periodic changes in the environment, such as light and food are integrated across several well-known central neural and peripheral systems. Biological variation in internal signals, both internal hormonal milieu (HE) and circadian clock genes (e.g. BMAL1) govern the expression of epigenetic enzymes. This output signal acts in a tissue- and cell-specific manner, on epigenetic enzymes (e.g. DNMT3a and TET1/2) and alters the epigenomic landscape. The genome-wide variation in epigenetic modifications controls the timing of gene transcription that produces the period across ultradian, circadian and infradian timescales.

GLOSSARY BOX

Circadian: an endogenously maintained molecular biological rhythm that matches an environmental periodicity of about 24 hours.

Circannual: an endogenously maintained molecular biological rhythm that match an environmental periodicity of about 12 months.

Circaquadridian: an endogenous rhythm that has a period that approximates 4-5 days, such as the female rodent estrous cycle.

Developmental Epigenetics: the study of modifications in gene function during cellular determination and differentiation that affect gene transcription across the lifespan.

DNA methylation: an epigenetic mechanism that involves the addition of a methyl (CH3) group to the DNA template. The methyl group is covalently attached to nucleotide base pairs with a greater frequency on cytosine-guanine residues.

Epigenetic: cellular, tissue and/or phenotypic plasticity due to biochemical modifications to the genome template and/or chromatin structures. Epigenetic changes can induce modifications during critical developmental stages, during life-history transitions and transgenerational effects

Histone acetylation: an epigenetic modification that involved the process of adding an acetyl (Ac) group to a histone protein. Increased Ac on lysine histones induces a permissive state that facilitates gene transcription

Infradian: internal biological rhythms that match an environmental periodicity has a period greater than a day, but shorter than a month

Neuroendocrine: a nuclei, cell or peptide within the hypothalamus that maintains homeostasis and regulates a range of physiological systems including: energy balance, metabolism, reproduction, osmolarity and body temperature.

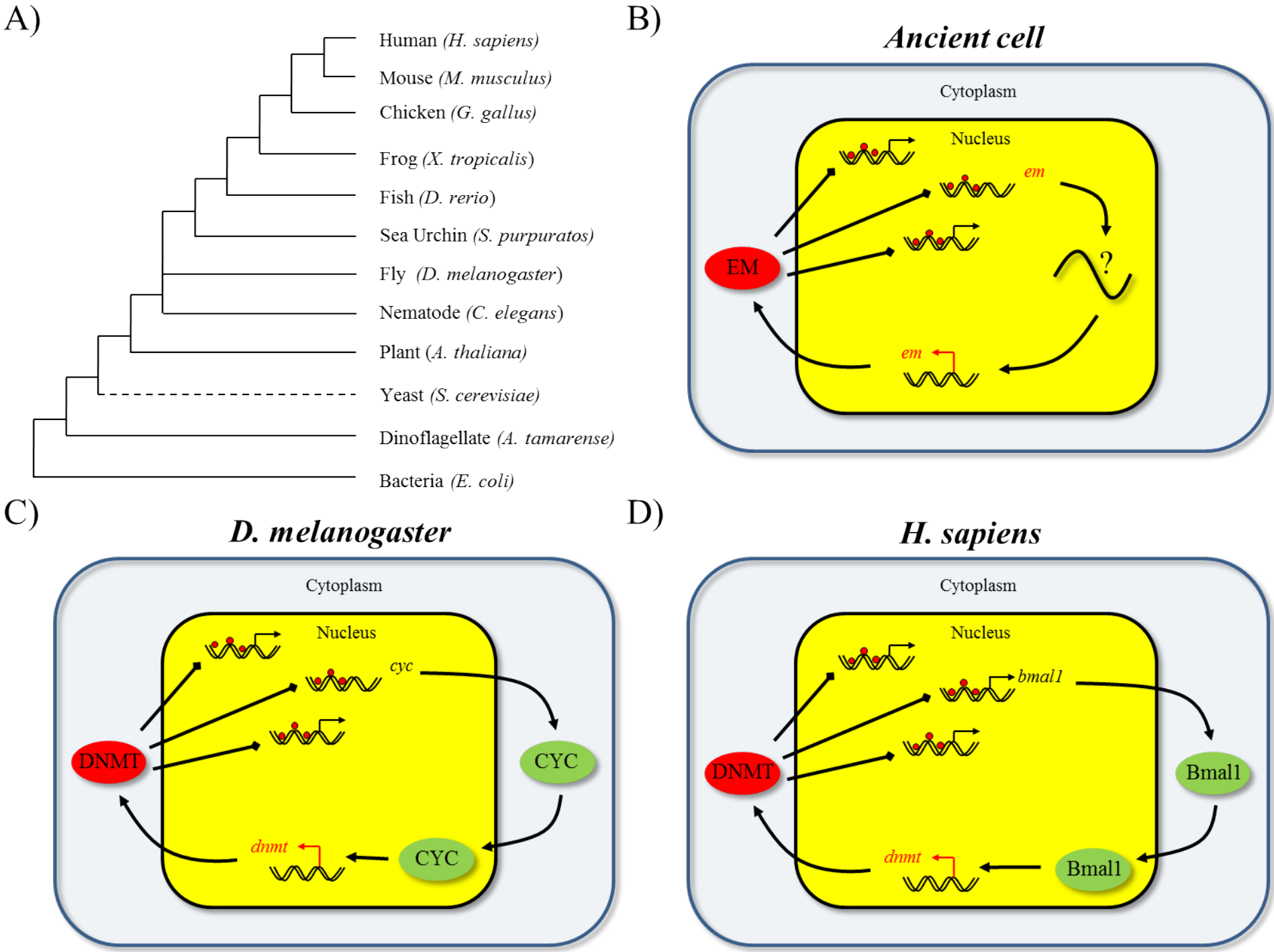
Pacemaker: anatomical or cellular region that functions to sustain an endogenous oscillation and entrain other oscillators

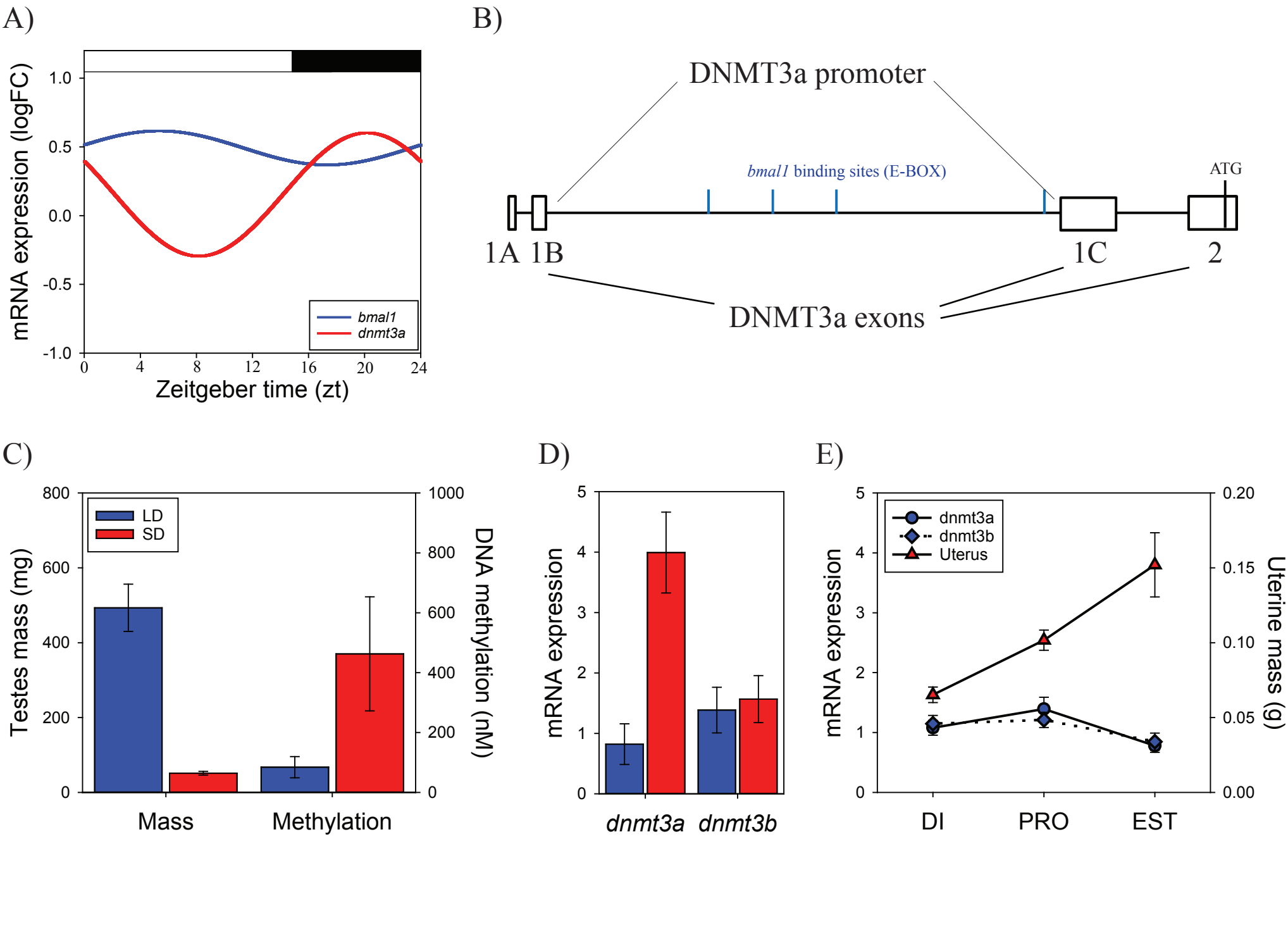
Photoperiod: the time of light in a daily light-dark cycle. The use of changes in the day length on an annual basis to regulate seasonal processes is referred to as photoperiodism.

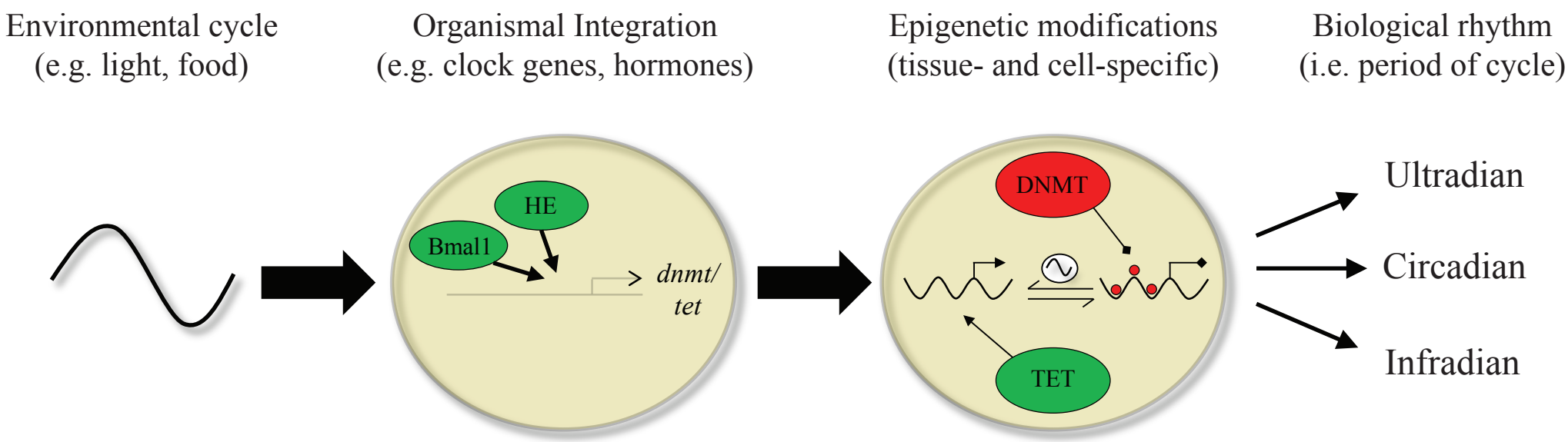
Rhythmic epigenetics: the study of timing and oscillations of modifications in gene function that impart heritable and lifespan changes independent of alterations in genome sequence.

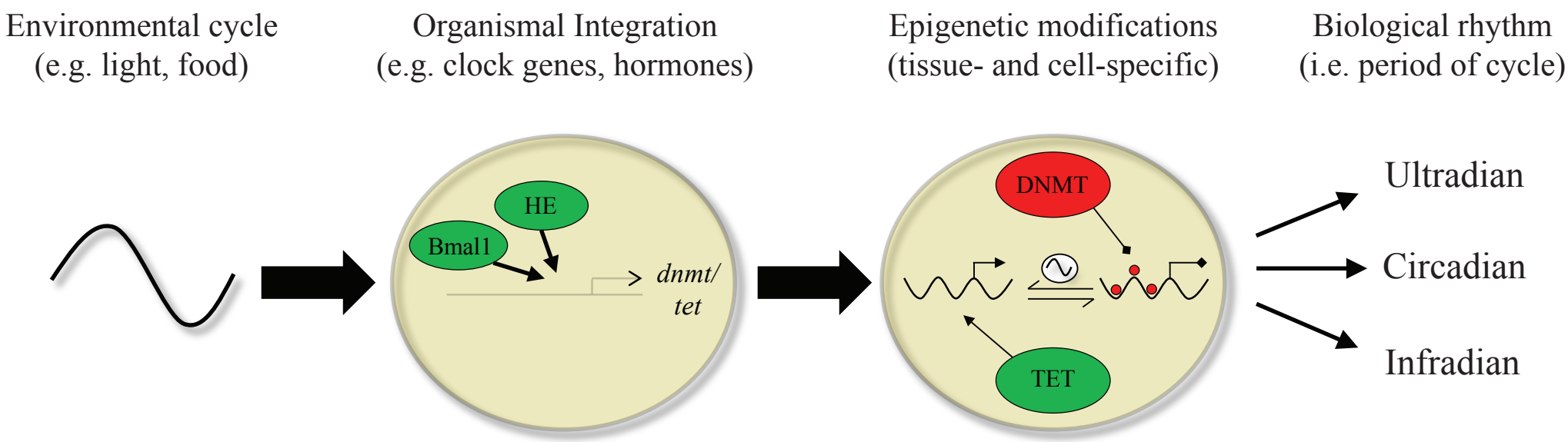
Transgenerational epigenetics: the study of heritable modifications in gene function that occur independently of changes to genome sequence.

Ultradian: internal biological rhythms that has a period less than 24 hours or oscillates more than once per day.


A)
Human (H. sapiens)
Mouse (M. musculus)
Chicken (G. gallus)
Frog (X. tropicalis)
Fish (D. rerio)
Sea Urchin (S. purpuratos)
Fly (D. melanogaster)
Nematode (C. elegans)
Plant (A. thaliana)
Yeast (S. cerevisiae)
Dinoflagellate (A. tamarense)
Bacteria (E. coli)
B)
Ancient cell
Cytoplasm
Nucleus
em
EM
?
em
C)
D. melanogaster
Cytoplasm
Nucleus
cyc
DNMT
CYC
dnmt
CYC
D)
H. sapiens
Cytoplasm
Nucleus
bmal1
DNMT
Bmal1
dnmt
Bmal1


A)
mRNA expression (logFC)
1.0
0.5
0.0
-0.5
-1.0
0
4
8
12
16
20
24
Zeitgeber time (zt)
bmal1
dnmt3a
B)
DNMT3a promoter
bmal1 binding sites (E-BOX)
ATG
1A
1B
1C
2
DNMT3a exons
C)
Testes mass (mg)
DNA methylation (nM)
LD
SD
800
600
400
200
0
1000
800
600
400
200
0
Mass
Methylation
D)
mRNA expression
5
4
3
2
1
0
dnmt3a
dnmt3b
E)
mRNA expression
Uterine mass (g)
dnmt3a
dnmt3b
Uterus
5
4
3
2
1
0
0.20
0.15
0.10
0.05
0.00
DI
PRO
EST


Environmental cycle
(e.g. light, food)
Organismal Integration
(e.g. clock genes, hormones)
Epigenetic modifications
(tissue- and cell-specific)
Biological rhythm
(i.e. period of cycle)
HE
Bmal1
dnmt/
tet
DNMT
TET
Ultradian
Circadian
Infradian


Environmental cycle
(e.g. light, food)
Organismal Integration
(e.g. clock genes, hormones)
Epigenetic modifications
(tissue- and cell-specific)
Biological rhythm
(i.e. period of cycle)
HE
Bmal1
dnmt/
tet
DNMT
TET
Ultradian
Circadian
Infradian

Outstanding Questions Box

- The model outlined herein predominantly examines molecular rhythms in metazoan species, how are epigenetic enzymes regulated in protozoan species and how do these enzymes regulate rhythmic processes?
- Do mutations in epigenetic enzymes (e.g. *dnmt3a*) altered ultradian, circadian or infradian period?
- BMAL binds *dnmt3a* in a circadian manner, but does the *bmal* promoter/intron/exon or other clock genes exhibit circadian variation in methylation?
- Do epigenetic enzymes exhibit ultradian rhythms?
- What is the relative contribution of other modifications, including RNA methylation, protein methylation for timing rhythms in gene transcription?
- Do DNA methyltransferase enzymes display rhythmic binding on non-clock gene promoter/intron/exons?
- Similarly, do rhythmic histone acetyltransferase and deacetyltransferase enzymes exhibit preferential binding on specific histone proteins?
- Do rhythmic transcription factors recruit epigenetic enzymes to genomic/histone targets or do epigenetic modifications facilitate recruitment?
- Males and females integrate environmental cues in a sex-dependent manner, is this reflected in the initiation of cell- and tissue-specific epigenetic enzyme expression and does this impact downstream genomic and/or chromatin regions?
- Are rhythms in the epigenomic landscape inheritable?
- Are rhythmic epigenomic modifications involved in developmental programming?

Trends Box

Biological rhythms are present across diverse life forms and occur at genomic, epigenomic, molecular, physiological, neural and behavioural levels.

Specific epigenetic enzymes are regulated by endogenous molecular rhythms and hormonal signals that in turn induce oscillatory patterns across multiple timescales.

Daily and seasonal rhythms in epigenetic enzymes may significant consequences for the epigenomic landscape, timing molecular processes such as gene transcription and exhibit tissue- and cell-specific modifications.

DNA methylation in particular is an evolutionary ancient mechanism that could have short- and long-term impacts on timing biological rhythms; disruptions in epigenetic oscillations may consequences for health and wellness.