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

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20 **Abstract**

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

30

31 **Introduction**

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

46 In this Opinion, the proposition that epigenetic modifications are an evolutionary ancient and
47 essential component of the genomic regulation of biological rhythms is proposed. **The central premise**
48 **of the epigenetic clock model is that the timing, or rhythmic epigenetic modifications impacts normal**
49 **and pathological genomic states across the lifespan and subsequent generations. The evidence is**
50 **derived from data that support a link between circadian clock genes and hormonal pathways, and the**
51 **cell- and tissue specific expression of enzymes involved in DNA methylation and histone acetylation.**
52 First, the various rhythmic periods over which biological rhythms have been observed is reviewed,
53 followed by an overview of the epigenetic modifications that have mapped onto such timescales. Next,
54 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,

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

82

83 Core molecular mechanisms that control epigenetic modifications

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

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

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

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

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

134

135 Evidence for rhythms in epigenetic modifications

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

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

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

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

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

184

185 Framework for an Epigenetic Clock Model

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

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

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

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

223 between photoperiodic regulation seasonal rhythms and DNA methylation in plants [43], invertebrate
224 [49] and vertebrate species [45,46]. It remains possible that circannual rhythms generated and
225 maintained by epigenetic modifications could be driven, at least in part, by annual changes in circadian
226 clock genes. However, the evidence that SCN-lesions in ground squirrels [17] and molecular
227 oscillatory patterns in gene expression in reindeer [16] together indicate that circannual rhythms in
228 epigenetic modifications may be independent of circadian timing mechanisms. Therefore, the long-
229 term internal representation of circannual time could be driven by tissue and cell-specific epigenetic
230 modifications. Here, the annual DNA methylation of key genomic regions would be proposed to
231 approximate 365 days and coordinate the seasonal timing of gene transcription required for the
232 expression of circannual rhythms in physiology and behaviour. Epigenetic modifications are
233 hypothesized to be integral components of the endogenous long-term timer and entrained by seasonal
234 environmental cues (e.g. photoperiod) [50].

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

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

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

256 Concluding Remarks and Future Perspectives

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

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

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

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

290

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438

439

440 Figure Legends

- 441 Figure 1 Phylogenetic occurrence of biological rhythms in DNA methylation. A) Phylogenetic tree
 442 indicating the presence (solid line) and absence (dashed line) of DNA methylation. B) In a
 443 hypothetical evolutionary ancient cell, a negative feedback system could control biological
 444 rhythms in gene transcription. The detection of a periodical environmental stimulus,
 445 indicated by sinewave, is hypothesized to trigger the expression of an epigenetic modifier
 446 (EM; e.g. DNMT, HDAC). Increased EM expression then initiates a biochemical
 447 modification surrounding the DNA template; and governs the probability of gene
 448 transcription at targeted loci. In a simplified system, the EM provides negative feedback
 449 leading to the reduction in *em* transcription. Circadian rhythms in DNA methyltransferase
 450 (DNMT) expression provide daily epigenetic control over the timing of gene transcription.
 451 C) and D) are hypothetical schematic diagrams of epigenetic modifications via DNMT in
 452 *Drosophila melanogaster* and *Homo sapiens*, respectively. Cycle (CYC) and Aryl
 453 hydrocarbon receptor nuclear translocator-like BMAL1) proteins are two well described
 454 components of the molecular circadian clock. In these models, daily timing in gene
 455 transcription is provided by an oscillation in clock gene stimulation of DNMT, which in
 456 turn, increases DNA methylation in the promoter regions providing negative feedback
 457 leading to an inhibition of CYC/BMAL1 expression. The precise relationship between
 458 epigenetic enzymes (e.g. DNMT) and well-established circadian positive- and negative-
 459 feedback loops is yet to be determined.
 460
- 461 Figure 2 Current evidence for biological rhythms in DNA methylation and DNA methyltransferase
 462 enzyme expression. Siberian hamsters exhibit robust ultradian, circadian and infradian
 463 rhythms. (A) *dnmt3a* expression exhibits a circadian waveform that is in anti-phase with
 464 *bmal1* expression. The white-black bar across the top indicates lights on - lights off
 465 respectively. (B) Consite analyses of one *dnmt3a* promoter region located between exon 1b
 466 and 1c indicates a series of BMAL1 binding sites (i.e. E-Box) motifs. An circannual
 467 rhythm in testicular function (i.e. mass) is one control mechanism involved in the timing of
 468 seasonal reproduction. (C) In summer-long day (LD) phenotypes, hamsters are
 469 reproductively competent with large testes; upon exposure to winter-short days (SD) testes
 470 regress and reproduction is terminated. The global level of DNA methylation significantly
 471 increases in SD-regressed testes and is associated with the inhibition of spermatogenesis.
 472 (D) The seasonal rhythm in testicular DNA methylation is proposed to be under the control
 473 of DNMT3a as SD testes exhibit significantly greater levels of expression. Finally (E)
 474 *dnmt3a* expression also exhibits short-term estrous rhythms (i.e. circaquadradian), in
 475 particular, significant reductions occur in the uterus endometrium during the estrous cycle.
 476
- 477 Figure 3 Key Figure: schematic diagram of the Epigenetic Clock model for the regulation of
 478 biological rhythms. Periodic changes in the environment, such as light and food are
 479 integrated across several well-known central neural and peripheral systems. Biological
 480 variation in internal signals, both internal hormonal milieu (HE) and circadian clock genes
 481 (e.g. BMAL1) govern the expression of epigenetic enzymes. This output signal acts in a
 482 tissue- and cell-specific manner, on epigenetic enzymes (e.g. DNMT3a and TET1/2) and
 483 alters the epigenomic landscape. The genome-wide variation in epigenetic modifications
 484 controls the timing of gene transcription that produces the period across ultradian,
 485 circadian and infradian timescales.
 486

487 **GLOSSARY BOX**

488

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

491

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

494

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

497

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

500

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

504

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

509

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

513

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

516

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

520

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

523

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

526

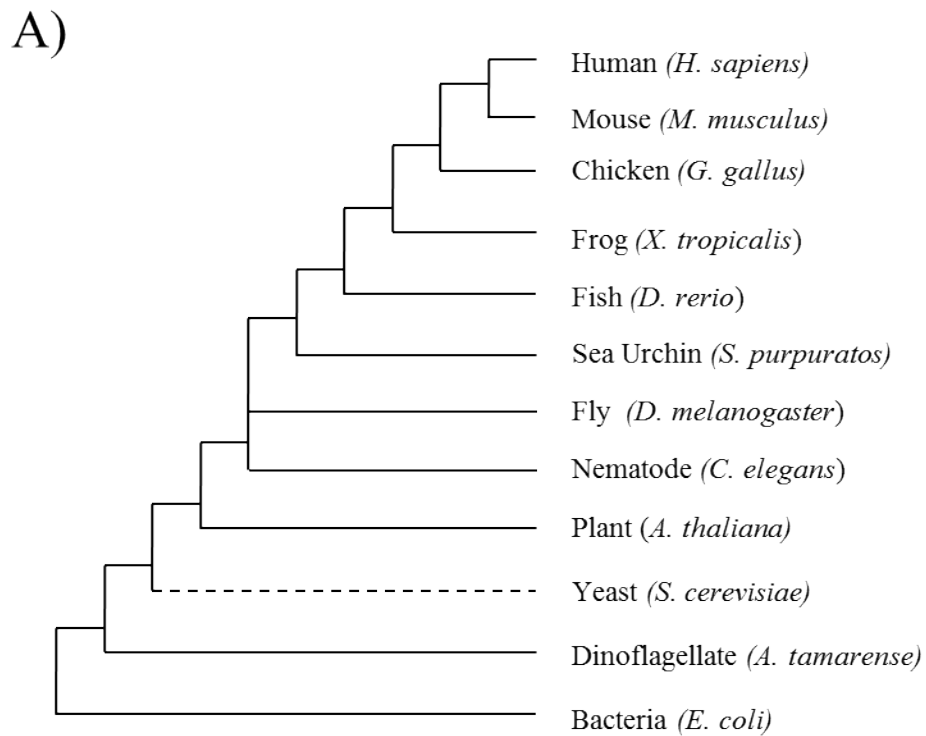
527 **Rhythmic epigenetics: the study of timing and oscillations of modifications in gene function that**
528 **impart heritable and lifespan changes independent of alterations in genome sequence.**

529

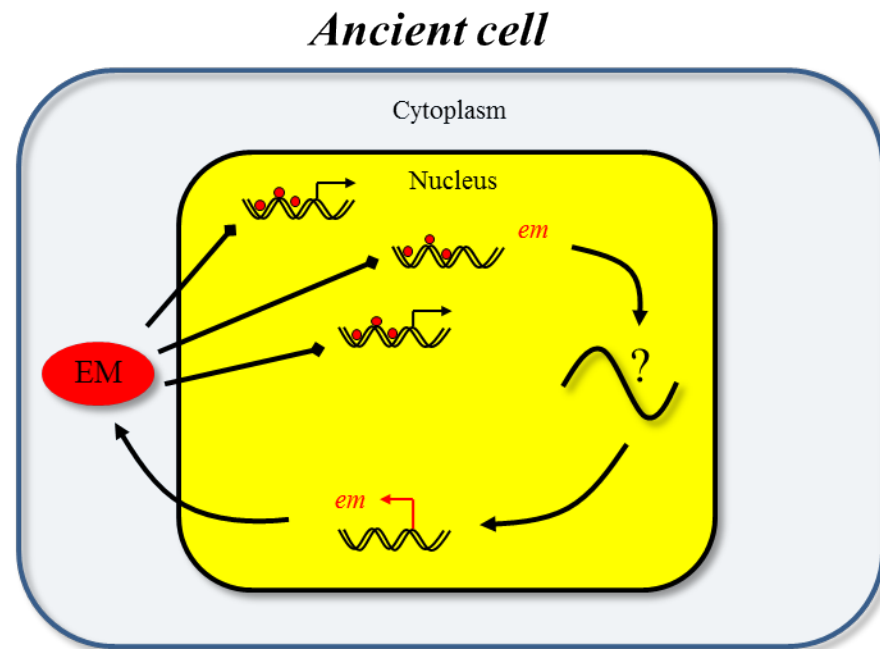
530 **Transgenerational epigenetics: the study of heritable modifications in gene function that occur**
531 **independently of changes to genome sequence.**

532

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

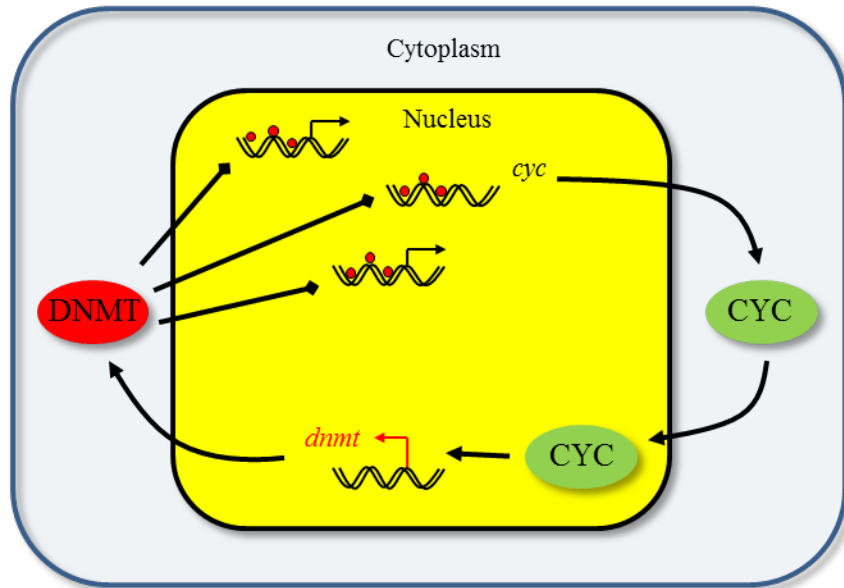


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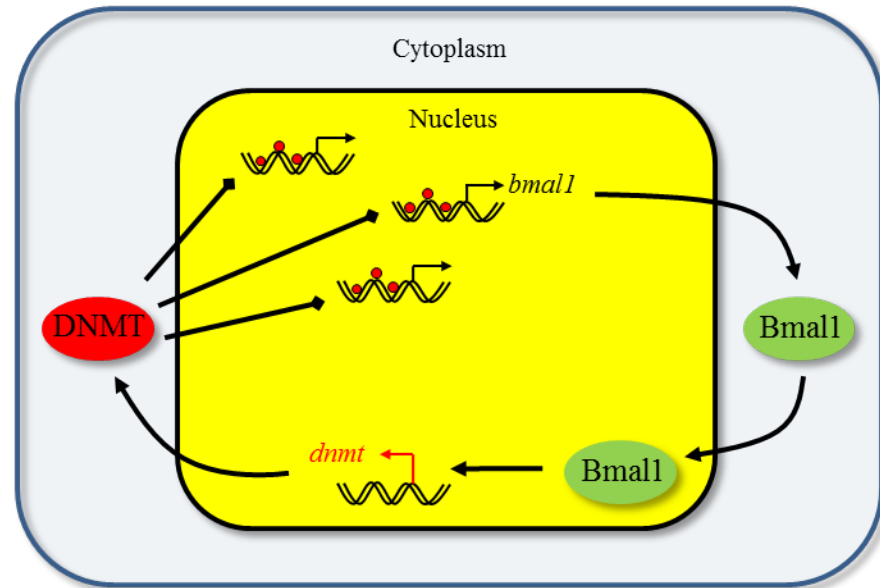
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D. melanogaster

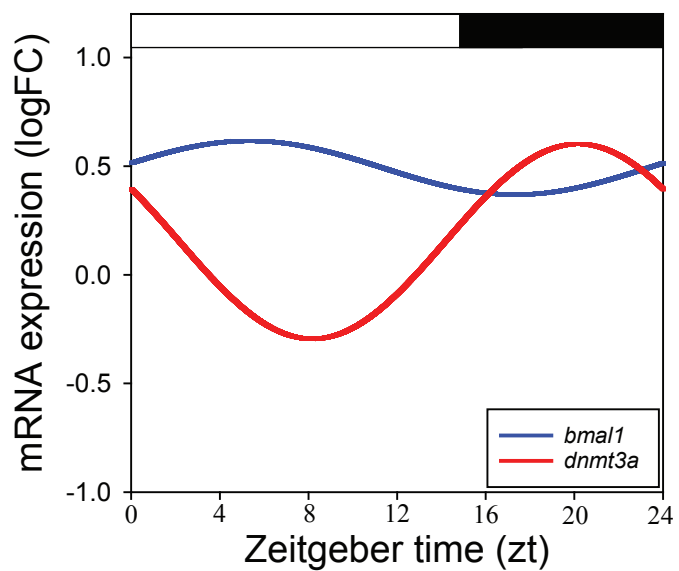


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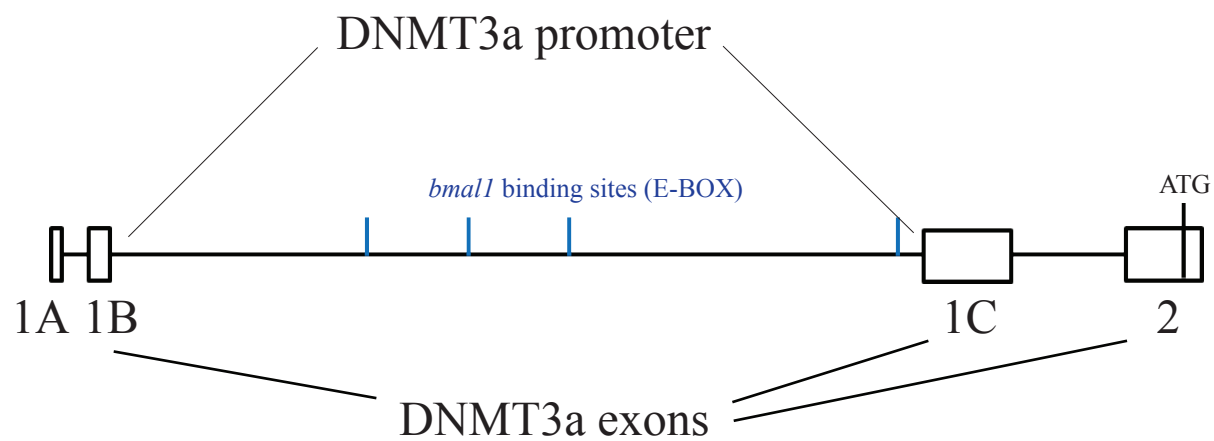
H. sapiens



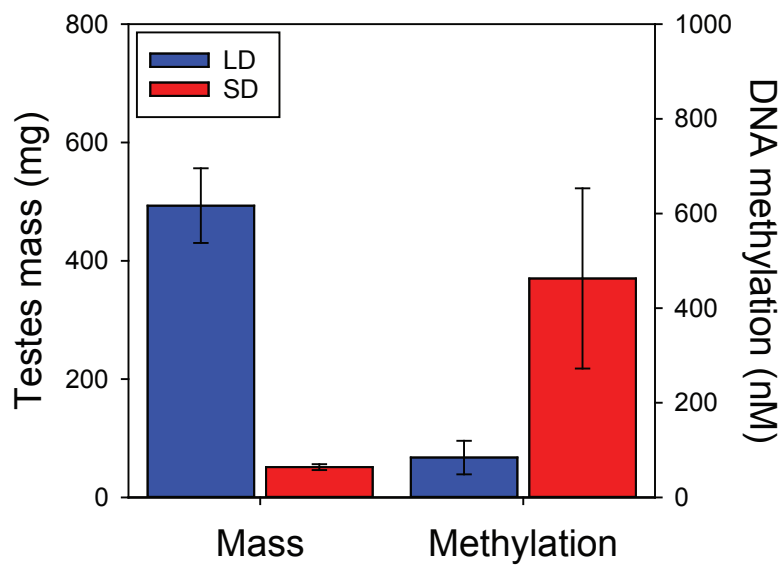
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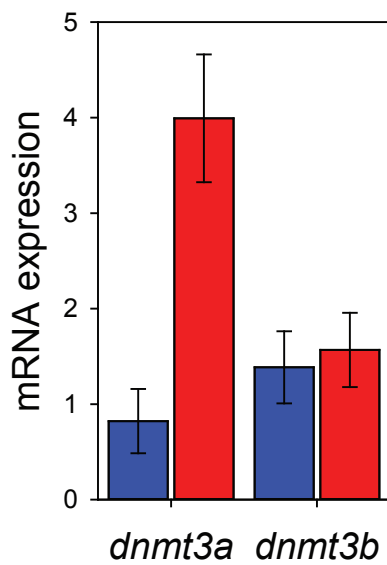
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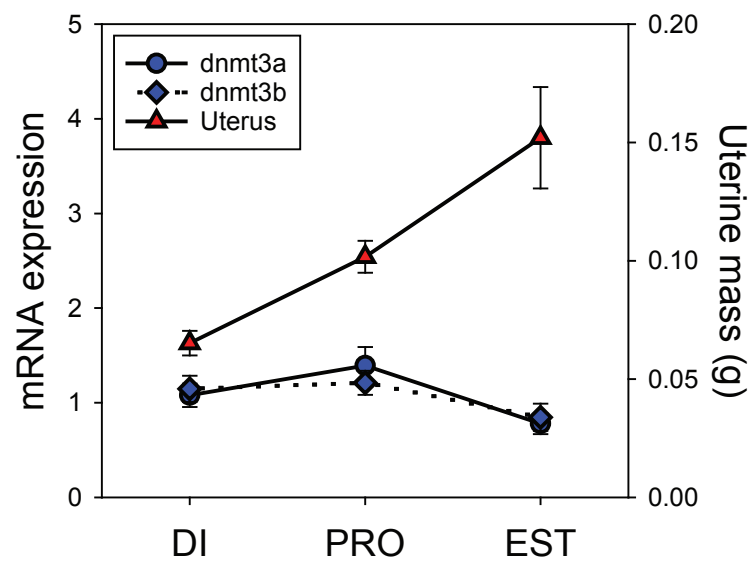
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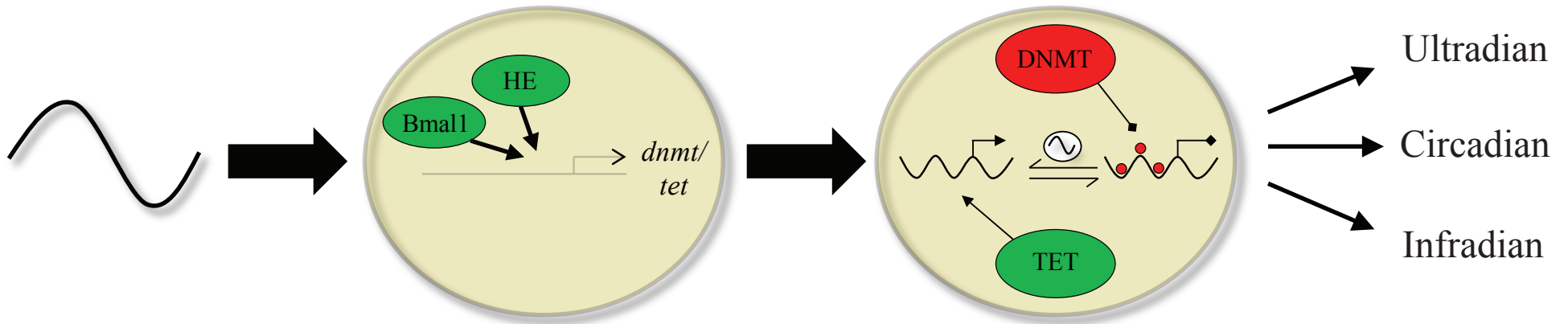


Environmental cycle
(e.g. light, food)

Organismal Integration
(e.g. clock genes, hormones)

Epigenetic modifications
(tissue- and cell-specific)

Biological rhythm
(i.e. period of cycle)

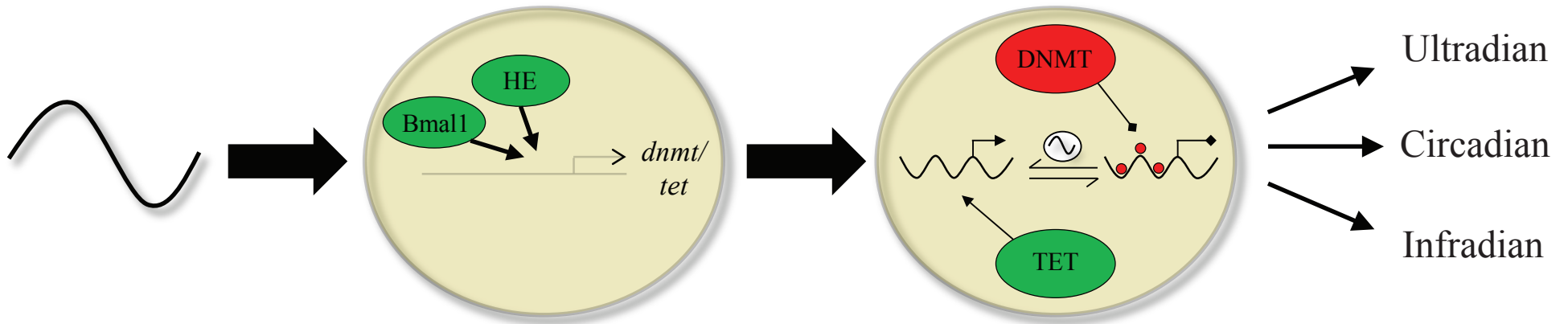


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Outstanding Questions Box

- 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.