

## Post-transcriptional controls - adding a new layer of regulation to clock gene expression.

Marie Cibois, Carole Gautier-Courteille, Vincent Legagneux, Luc Paillard

► **To cite this version:**

Marie Cibois, Carole Gautier-Courteille, Vincent Legagneux, Luc Paillard. Post-transcriptional controls - adding a new layer of regulation to clock gene expression.. Trends in Cell Biology, Elsevier, 2010, 20 (9), pp.533-41. 10.1016/j.tcb.2010.06.004 . inserm-00511205

**HAL Id: inserm-00511205**

**<https://www.hal.inserm.fr/inserm-00511205>**

Submitted on 24 Aug 2010

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



19 **Living organisms undergo biochemical, physiological and behavioural cycles with**  
20 **periods ranging from seconds to years. The cycles with intermediate periods rely on**  
21 **endogenous clocks that consist of oscillating gene expression. Our goal is to illustrate the**  
22 **modalities and specific functions of posttranscriptional controls of gene expression**  
23 **(exerted on pre-mRNAs and mRNAs) in biological clocks through two examples: the**  
24 **circadian clock and the vertebrate somitic segmentation clock, an embryonic clock with**  
25 **a period far below a day. We conclude that both uniformly and cyclically exerted**  
26 **posttranscriptional controls underpin the set-up of clock functions.**

27

## 28 **Rhythmic gene expression in oscillators**

29 Living organisms are submitted to periodic oscillations of biochemical, physiological  
30 and behavioural parameters that are named biological rhythms. For a given process, the  
31 periods of the cycles range from less than one second to several years (Box 1). The  
32 biorhythms are subdivided into circadian (period approximately equal to 24 hours), ultradian  
33 and infradian (respectively shorter and longer periods, See Glossary) [1].

34 The present review will focus on essentially two rhythms, the ultradian rhythm that  
35 underpins vertebrate somitic segmentation and the circadian rhythm. During vertebrate  
36 embryo elongation, somites (presumptive muscles and bones) periodically bud off the non-  
37 segmented, posterior mesoderm (presomitic mesoderm). This results in a repetitive  
38 organization all along the antero-posterior axis, which is referred to as somitic segmentation.  
39 The periodic emergence of somites relies on an autonomous ‘clock’ within the non-segmented  
40 mesoderm that oscillates with a period ranging from 30 minutes in zebrafish to 2 hours in  
41 mice [2].

42 In circadian rhythms, there also exists an internal clock that is able to free-run with a  
43 period of approximately 24 hours. This clock exists in multicellular organisms, but also in  
44 yeasts [3]. This autonomous clock is temporally ‘entrained’ by light–dark or temperature  
45 cycles [4-6]. In mammals, it is located in the suprachiasmatic nucleus (SCN), a group of

46 hypothalamic neurons. Neuronal connections between the retina and the SCN explain the  
47 entrainment by light-dark cycles, which is evidenced among others by the resetting of the  
48 clock when light-dark cycles are shifted by some hours (in experimental conditions or  
49 following long-distance travels in humans) [4,5].

50         The mammalian circadian clock relies on eight proteins that are cyclically expressed in  
51 the SCN (Figure 1A): Clock [7], Bmal1 (Mop3) [8], Per1, Per2 and Per3 [9], Cry1 and Cry2  
52 [10], and Rev-Erb $\alpha$  [11]. The Clock-Bmal1 complex controls the expression of several genes  
53 at the transcription level, among which *Period* (*Per1* to *Per3*), *Cryptochrome* (*Cry1* and  
54 *Cry2*), and *Rev-Erb $\alpha$* , through its association with E-box elements. The Per-Cry protein  
55 complexes interact with and inhibit Clock-Bmal1, and Rev-Erb $\alpha$  inhibits the transcription of  
56 *Bmal1*. These two transcriptional feedback loops are responsible for the oscillations of Clock-  
57 Bmal1 activity that themselves account for the circadian expression of the clock outputs  
58 (Figure 1A) [4,5]. Several additional factors that modulate the mammalian circadian clock  
59 were recently identified by RNAi or proteomic approaches [12,13]. The circadian and the  
60 segmentation (Box 2) clocks both are set-up by transcriptional negative-feedback loops [2,14-  
61 18].

62         In addition to transcriptional loops, the control of the degradation of the proteins  
63 encoded by the clock genes determines their amounts in both clocks [17,19-21]. Several  
64 posttranslational modifications determine the activity and the stability of clock proteins [19].  
65 Together, they represent a second layer of gene regulation in clock functions. A third layer of  
66 gene regulation must now be considered when investigating biological rhythms (Figure 1B).  
67 This layer, collectively referred to as posttranscriptional controls, encompasses all the  
68 regulations that are exerted at the RNA level (Box 3). They are mediated by ribonucleoproteic  
69 particles that include RNA-binding proteins (RNA-BPs) and non-coding RNAs, especially  
70 microRNAs (miRNAs) [22-24]. Their contributions in essential clock functions are an  
71 emerging and important field of study.

72

### 73 **Circadian rhythms as a paradigm for dynamic posttranscriptional controls**

74 The first evidence for posttranscriptional controls in circadian rhythms came from  
75 pioneering work in the fruitfly *Drosophila* [25]. Since, oscillating mRNA stability during the  
76 circadian cycle was also demonstrated in the mammalian core pacemaker (Figure 2). The  
77 stabilities of *Per2* and *Cry1* mRNAs vary during the cycle in mice, and, together with  
78 oscillating transcription, this results in rhythmic expression [26,27]. Woo and colleagues  
79 found that the RNA-BPs Ptbp1 and Hnrpd are able to bind to the 3' untranslated regions of  
80 *Per2* and *Cry1* mRNAs, respectively, and cause their rapid degradation [26,27]. Furthermore,  
81 the levels of cytoplasmic Ptbp1 and Hnrpd oscillate during the circadian clock and are  
82 correlated with target mRNA decay rates. In synchronized cultured cells, the oscillations of  
83 *Per2* and *Cry1* mRNAs were affected when the levels of Ptbp1 and Hrpd were reduced by  
84 RNAi. Together, these results suggest that oscillating amounts of cytoplasmic RNA-BPs may  
85 be responsible for the oscillating stability of target mRNAs that in turn determines their  
86 oscillating expression [26,27].

87 Rhythmic translation is another strategy to achieve cyclic expression of clock genes in  
88 the SCN, as demonstrated for *Per1* mRNA (Figure 2). The RNA-BP Rbm4 is cyclically  
89 expressed in-phase with *Per1*. It is able to bind to *Per1* mRNA and to stimulate its translation.  
90 Hence, translational stimulation by Rbm4 synergizes with transcriptional controls to amplify  
91 the level of *Per1* oscillations [28]. Interestingly, only Rbm4 protein, but not *Rbm4* mRNA, is  
92 cyclically expressed, indicating that Rbm4 expression is itself controlled at a translational or  
93 posttranslational (protein degradation) level [28]. It is not known whether Rbm4 is required  
94 for circadian rhythms in whole mammalian organisms, but manipulating its level in cultured  
95 mammalian cells or in *Drosophila* affects circadian oscillations [28,29].

96 In addition to RNA-BPs, microRNAs (miRNAs) also control several mRNAs within  
97 the circadian pacemaker (Figure 2). miRNAs affect both mRNA stability and translation [22].  
98 In animals, the interactions between miRNAs and target mRNAs are mediated by limited

99 sequence conservation. A miRNA can have several mRNA targets that are difficult to  
100 identify, although considering preferential evolutionary conservation improved the capacity to  
101 predict miRNA-mRNA interactions in silico [30]. Cheng and colleagues [31] showed that the  
102 miRNAs miR-219 and miR-132 have a circadian expression in the SCN, and they identified  
103 several potential mRNA targets. *Per2* protein is overexpressed upon treatment with an  
104 antisense (antagomir) oligonucleotide against miR-132, which is consistent with miR-132  
105 downregulating the translation of *Per2* mRNA. Furthermore, circadian period length and  
106 light-dependent clock resetting are altered in the absence of miR-219 and miR-132  
107 respectively [31].

108         The SCN emits circadian signals to other regions of the brain, including the pineal  
109 gland. This gland synthesizes melatonin during the night and this circulating hormone relays  
110 the circadian rhythm to the peripheral organs. Arylalkylamine N-acetyltransferase (*Aanat*) is  
111 cyclically expressed in the pineal gland and is the rate-limiting enzyme in melatonin  
112 synthesis. Its expression is controlled at several levels, including mRNA stability and  
113 translation (Figure 2). The 3' untranslated region of *Aanat* mRNA contains a destabilizing  
114 element, and three rhythmically expressed RNA-BPs (*Hnrnpr*, *Hnrnpl*, *Syncrip*) are able to  
115 bind to this element and may play a role in the rhythmic degradation of *Aanat* mRNA [32]. In  
116 addition, *Aanat* mRNA is translated through an IRES (internal ribosome entry site), and  
117 *Syncrip* is able to bind to that IRES and stimulate *Aanat* mRNA translation. The oscillations  
118 of *Syncrip* protein during circadian cycles result in in-phase oscillations of *Aanat* mRNA  
119 translation, and manipulating the level of *Syncrip* impacts melatonin production in  
120 pinealocytes [33]. It is probable that the oscillations of *Hnrnpr*, *Hnrnpl* and *Syncrip* are  
121 themselves controlled by circadian cues sent by the SCN, but how this is achieved is unknown  
122 (Figure 2).

123         In addition to brain, most mammalian organs contain autonomous clocks that are  
124 entrained by cues emitted by the master clock [34], and posttranscriptional controls might  
125 operate in these peripheral clocks too. A comprehensive microarray experiment revealed

126 ultradian rhythmic expression of several genes in mouse liver [35]. This might indicate some  
127 ultradian clock, but an alternative cause could be mRNA degradation. If genes are transcribed  
128 following circadian rhythms and the corresponding mRNAs are degraded following a  
129 circadian, out-of-phase, rhythm, the mRNA levels might oscillate with a period of 12 hours  
130 [35].

131 A function for oscillating mRNA stability in circadian rhythm has also been described  
132 in plants. A microarray screening in *Arabidopsis thaliana* identified two mRNAs whose  
133 stabilities oscillate with a period of 24 hours. Disruption of the pathway responsible for the  
134 rapid degradation of these mRNAs in the afternoon alters the oscillations of these mRNAs in  
135 correlation with an altered circadian rhythm at the whole-plant level, indicating a link  
136 between circadian rhythms in plants and specific mRNA decay [36].

137

### 138 **Clues for the importance of posttranscriptional controls in biological rhythms**

139 How widespread are posttranscriptional controls of gene expression in biological  
140 rhythms? A rough estimate is provided by identifying factors that control gene expression at  
141 the posttranscriptional level and that display a rhythmic circadian expression. This is the case  
142 for several miRNAs in the plant *Arabidopsis thaliana* [37], fly heads [38] and mouse retinas  
143 [39].

144 Several examples of oscillating RNA-BPs have also been reported, in addition to the  
145 factors described in the previous section. In the green alga *Chlamydomonas reinhardtii*, the  
146 capacity of the RNA-binding complex CHLAMY1 to bind to target mRNAs follows a  
147 circadian rhythm [40]. CHLAMY1 comprises two subunits that both are RNA-BPs.  
148 Experimentally manipulating the level of either of these two subunits strongly interferes with  
149 the circadian rhythm, suggesting that these two proteins are at the heart of the circadian clock  
150 in this species [41]. The *Chlamydomonas* clock is entrained by temperature cycles, and both  
151 subunits of CHLAMY1 are involved in temperature integration [42]. In rats, the RNA-BP  
152 Mbnl2 (Muscleblind 2) that is involved in alternative splicing of pre-mRNA has an oscillatory

153 expression in the pineal gland [43]. Finally, Nocturnin, a poly(A) ribonuclease (that causes  
154 mRNA decay and translational repression by removing the poly(A) tails, see Box 3), is  
155 cyclically expressed in the retina [44]. Surprisingly, mice in which the *Nocturnin* gene has  
156 been inactivated display normal circadian rhythms and expression of clock genes (but altered  
157 lipid metabolism or uptake) [45]. Hence, factors that control mRNA fate and display a  
158 rhythmic expression pattern can be divided into two groups: those that directly influence the  
159 clock, and those, like Nocturnin, that represent its readouts.

160 An additional clue to estimate the extent of translational controls in biological rhythms  
161 is to compare the levels of cycling proteins with their corresponding mRNAs. Systematic  
162 comparison of the transcriptome and the proteome of mouse liver showed that only half of the  
163 genes that exhibit rhythmic protein expression also exhibit rhythmic mRNA expression [46].  
164 Interestingly, circadian variations in protein isoforms were also reported by these authors,  
165 which are consistent with circadian modifications of alternative splicing [46]. The strong  
166 discrepancies between transcriptome and proteome data suggest prevalent translational and/or  
167 posttranslational (protein degradation) controls of cyclic gene expression in the circadian  
168 clock.

169

#### 170 **One step forward: how are cyclic posttranscriptional controls generated?**

171 As seen above, cyclical posttranscriptional controls are exerted on several mRNAs and  
172 in several physiological systems. In some already discussed cases, the factors involved in  
173 RNA regulations are uniformly expressed, but their activity or subcellular localisations  
174 oscillate [26,27,41]. The mechanisms underlying these oscillations are unknown.

175 The factors controlling mRNA fate may also themselves be cyclically expressed,  
176 owing to a cyclical transcriptional regulation, as demonstrated for miR-219 (see Figure 2)  
177 [31], but also owing to posttranscriptional negative-feedback loops. In *Neurospora crassa*,  
178 FRQ and FRH proteins form the FFC complex, which is able to recruit the RNA exosome (a  
179 multi-subunit complex involved in mRNA degradation [47]) to *frq* mRNA, and to thereby



180 cause its degradation. Together with the capacity of FFC to repress the transcription of *frq*  
181 gene, this posttranscriptional negative-feedback loop achieves circadian oscillations in *N.*  
182 *crassa* [48]. In *Arabidopsis thaliana*, *AtGRP7* and *AtGRP8* are two RNA-BPs with a  
183 circadian expression. *AtGRP7* overexpression ablates circadian expression of *Atgrp7* and  
184 *Atgrp8* mRNAs [49]. Both proteins are able to bind to their own pre-mRNAs and direct their  
185 splicing pathways towards mRNA isoforms that contain a premature termination codon.  
186 These isoforms are rapidly degraded by the non-sense-mediated mRNA decay (NMD)  
187 pathway (see Box 3). Consequently, *AtGRP7* and *AtGRP8* negatively auto-regulate and cross-  
188 regulate their synthesis [50,51]. This mechanism very probably ensures a cyclical stability of  
189 the mRNAs encoding *AtGRP7* and *AtGRP8*, which contributes to their circadian oscillations.

190 In mammals, the RNA-BPs Rbm4 and Syncrip display oscillating expressions [28,33].  
191 It is tempting to speculate that these oscillations result from negative auto-regulations similar  
192 to plant *AtGRP7* and *AtGRP8* or *N. crassa* FRQ. Indeed, several mammalian RNA-BPs  
193 negatively regulate their own synthesis. PTBP1 and PTBP2 regulate the splicing of their own  
194 respective pre-mRNAs and promote the skipping of an exon that results in an NMD sensitive  
195 transcript [52,53]. They also cross-regulate each other through this splicing event [54,55].  
196 Similarly, the RNA-BP Celf2 negatively autoregulates its synthesis by inhibiting the splicing  
197 of its own pre-mRNA [56]. Whether these negative auto-regulations of RNA-BPs generate  
198 oscillations, and how these putative posttranscriptional negative-feedback loops are  
199 interconnected with the master transcriptional loop, have not been tested in mammals.

200

201 **Posttranscriptional controls do not need to be cyclically exerted to play a role in**  
202 **biological rhythms.**

203 Transcriptional negative-feedback loops result in successive activations and  
204 repressions of gene promoters. When transcription is shut off, mRNAs decay following  
205 exponential kinetics. If the decay of a given mRNA is sufficiently rapid (short half-life)  
206 relative to the period of transcriptional oscillations, then almost complete removal of the

207 mRNA will occur before transcription resumes. This situation produces oscillations of mRNA  
208 of maximum amplitude. However, if the transcription resumes before the mRNA is  
209 completely degraded, then the amplitudes of the mRNA oscillations are reduced or the  
210 oscillations are damped and, at the extreme of very stable mRNAs, completely disappear.  
211 Therefore, rapid mRNA degradation is required to convert switches between active and  
212 inactive transcription into oscillatory amounts of the corresponding mRNAs. One could  
213 predict therefore that rapid and uniform mRNA decay is instrumental in the generation of  
214 short-period (ultradian) biorhythms, and this prediction has at least been partially confirmed  
215 in the case of vertebrate somitic segmentation clock.

216         The period of the somitic segmentation clock is comprised between 30 minutes and 2  
217 hours [2]. Within one period, the amounts of several tens of mRNAs oscillate [57]. It takes no  
218 more than a few minutes to have a cyclic mRNA completely degraded, indicating very short  
219 half-lives. The data demonstrating the occurrence of posttranscriptional controls in somitic  
220 segmentation are summarized in Table 1.

221         The expression pattern of *Lunatic Fringe* (*Lfng*, a modulator of Notch signalling, one  
222 of the pathways required for segmentation) has been described in mice. In situ hybridizations  
223 were made with both an exonic probe to reveal the mRNA and an intronic probe to reveal  
224 sites of active transcription. The staining patterns with these two probes were very similar,  
225 demonstrating that *Lfng* mRNA is degraded virtually as rapidly as the *Lfng* introns [58]. Since  
226 splicing occurs co-transcriptionally, and excised introns are very rapidly degraded, these data  
227 demonstrate the remarkable instability of *Lfng* mRNA.

228         Reporter genes also showed that mRNA degradation is required to achieve the  
229 dynamic expression pattern of the clock genes. In Zebrafish, a GFP reporter controlled by the  
230 *Her1* promoter (an oscillating component of the core clock) accumulates in the presomitic  
231 mesoderm owing to its high stability, suggesting *a contrario* the rapid decay of the  
232 endogenous mRNA [59]. In *Xenopus* transgenic embryos, a characteristic striped expression  
233 pattern of *Hairy2a* and *Bowline*, two genes downstream of the clock, is recapitulated by

234 reporter mRNAs only if they contain a destabilizing element in their 3' untranslated regions  
235 (3'UTR) [60,61]. Taking as evidence for rapid mRNA degradation the capacity of a 3'UTR to  
236 confer upon a reporter GFP gene a striped pattern of expression, several chick or mouse clock  
237 mRNAs can be considered as unstable (Table 1 [60]). More recently, an approach combining  
238 *in ovo* electroporation and an inducible promoter showed that chick *Lfng* mRNA is  
239 destabilized by means of its 3'UTR [62].

240         What happens to segmentation if the rapid degradation of the cyclic mRNAs is  
241 impaired? Computational models of the zebrafish segmentation clock predict that the  
242 oscillations of the core clock genes are sustained only if the corresponding mRNA and  
243 proteins are unstable [18,63], but this was not experimentally tested at the mRNA level. In  
244 Zebrafish, the '*tortuga*' mutant shows an altered pattern of expression of *Her1* with impaired  
245 oscillations that is consistent with mRNA stabilisation [64]. The corresponding wild-type  
246 gene product may therefore be responsible for the rapid decay of *Her1* mRNA. This gene has  
247 not been identified. In *Xenopus*, the RNA-BPs Celf1 and Fxr1p regulate the stability and/or  
248 the translation of bound mRNAs, and knock-down of these proteins causes segmentation  
249 defects [65,66]. This suggests that these proteins have to bind and control a subset of mRNAs  
250 for correct segmentation to occur. The mRNA encoding Su(H), that is involved in Notch  
251 signalling in the segmentation clock, was identified as a target of Celf1. Specifically, a  
252 functional interaction between Celf1 and *Su(H)* mRNA is required for both the degradation of  
253 this mRNA and somitic segmentation [67]. Together, these data show that uniform mRNA  
254 regulation plays a key role in oscillations of the segmentation clock.

255         Continuous posttranscriptional controls were also described in the circadian clock. The  
256 expression of the microRNAs miR-192 and mi-R194 in cultured mammalian cells [68], miR-  
257 122 in mouse liver [69] or *bantam* in fly heads [70] apparently does not follow a circadian  
258 cycle (although miR-122 is cyclically transcribed but remains at approximately constant  
259 levels due to a long high-life [69]). All these miRNAs continuously downregulate identified  
260 target mRNAs encoding proteins involved in the circadian clock, and manipulating their

261 levels modifies the period and/or amplitude of circadian oscillations [68-70]. Other examples  
262 are given by *Per1* and *Per3* mRNAs that are uniformly unstable in NIH3T3 cells and  
263 transgenic mice, respectively [71,72]. The circadian oscillations of *Per3* mRNA are strongly  
264 modified when its mRNA degradation element is deleted [71]. Hence, constant  
265 posttranscriptional repression may be required in some instances to achieve optimal circadian  
266 oscillations in addition to cyclical posttranscriptional controls of gene expression.

267

### 268 **Concluding remarks and future directions**

269 The comparison of the segmentation and circadian clocks paves the way for future  
270 researches (Box 4). Both mRNA degradation and translation, mediated by RNA-BPs and  
271 miRNAs, have recognized functions in the circadian clock. In several instances, translational  
272 efficiency and mRNA degradation oscillate in the circadian clock, and these oscillations fully  
273 contribute to the clock. By contrast, the only known mode of posttranscriptional control in the  
274 segmentation clock is constant mRNA degradation. In fact, we might simply lack data  
275 concerning the different modes of posttranscriptional controls in the segmentation clock.  
276 Using the circadian clock as a paradigm for posttranscriptional controls in clocks, we  
277 recommend that the various modes of oscillating posttranscriptional controls should be  
278 carefully investigated in the segmentation clock. Furthermore, most but not all known modes  
279 of posttranscriptional controls were described in the circadian clock. Specifically, we know  
280 nothing about the subcellular localization and the putative localized translation of the mRNAs  
281 encoding factors of the clock. It might be of interest to investigate these points in the  
282 regulation of mammalian circadian clock considering their recognized importance in neurons  
283 [73].

284 Another question is whether there exist human diseases caused by posttranscriptional  
285 defects in clocks. Congenital vertebral malformations are often of genetic origin. Some of  
286 them were associated with mutations affecting genes of the segmentation clock, but the  
287 aetiology of most of them is unknown [74]. Factors involved in posttranscriptional regulations

288 in somitic segmentation, most of which were not identified, will be potential candidates for  
289 causing these syndromes. Also several human troubles arise from defects in the circadian  
290 clock, such as sleep disorders. Interestingly, fragile X patients suffer from sleep disorders  
291 [75]. This syndrome is a consequence of impaired expression of the RNA-BP FMR1, and  
292 *Fmr1* KO mice display an altered circadian rhythm [76]. Fragile X syndrome provides  
293 therefore a link between posttranscriptional controls, human pathology and the circadian  
294 clock, and it can be anticipated that this will not remain an isolated example.

295 A last issue is the extent of posttranscriptional controls in clocks. Several inactivations  
296 of gene encoding RNA-BPs were reported in mice. Some of them may be at the origin of  
297 circadian troubles that remained unnoticed up to now, and this would merit careful  
298 reinvestigation. For the RNA-BPs whose inactivations lead to clock troubles, the arising  
299 question will be the identity of the mRNAs that are normally associated with that protein and  
300 are deregulated upon its inactivation (and whose deregulation is responsible of the observed  
301 troubles). Recent technological breakthroughs allow some optimism concerning our capacity  
302 to ask that question. "CLIP" (Cross-linking and immunoprecipitation) allows the co-  
303 immunoprecipitation of RNA-BPs and associated RNAs [77]. Combined with next-generation  
304 sequencing, it permits the genome-wide identification of the RNAs bound by a protein  
305 ('CLIPseq') [78-80]. Maps of the interactions between miRNAs and mRNAs were drawn  
306 from Argonaute CLIPseq [81,82]. Together, these recent technologies will provide us with a  
307 genome-wide characterization of the network of posttranscriptional controls in virtually any  
308 cell type, including those subject to clock oscillations, and will allow us fully appreciating the  
309 extent of posttranscriptional controls in clocks.

310

## 311 **Acknowledgments**

312 We thank S. Hardy and H.B. Osborne for critical evaluation of the manuscript. V.L. is  
313 a staff member of the INSERM (Institut National de la Santé et de la Recherche Médicale).

314 Work in the authors' laboratory is supported by a grant from the Agence Nationale de la  
315 Recherche (ANR-07-JCJC-0097-01).

316

## 317 **References**

- 318 1 Schibler, U. and Naef, F. (2005) Cellular oscillators: rhythmic gene expression and  
319 metabolism. *Curr Opin Cell Biol* 17 (2), 223-229
- 320 2 Pourquie, O. (2003) The segmentation clock: converting embryonic time into spatial  
321 pattern. *Science* 301 (5631), 328-330
- 322 3 Eelderink-Chen, Z. *et al.* (2010) A circadian clock in *Saccharomyces cerevisiae*. *Proc*  
323 *Natl Acad Sci U S A* 107 (5), 2043-2047
- 324 4 Takahashi, J.S. *et al.* (2008) The genetics of mammalian circadian order and disorder:  
325 implications for physiology and disease. *Nat Rev Genet* 9 (10), 764-775
- 326 5 Gery, S. and Koeffler, H.P. (2010) Circadian rhythms and cancer. *Cell Cycle* 9 (6)
- 327 6 Rensing, L. and Ruoff, P. (2002) Temperature effect on entrainment, phase shifting,  
328 and amplitude of circadian clocks and its molecular bases. *Chronobiol Int* 19 (5), 807-  
329 864
- 330 7 Vitaterna, M.H. *et al.* (1994) Mutagenesis and mapping of a mouse gene, Clock,  
331 essential for circadian behavior. *Science* 264 (5159), 719-725
- 332 8 Bunger, M.K. *et al.* (2000) Mop3 is an essential component of the master circadian  
333 pacemaker in mammals. *Cell* 103 (7), 1009-1017
- 334 9 Zheng, B. *et al.* (2001) Nonredundant roles of the mPer1 and mPer2 genes in the  
335 mammalian circadian clock. *Cell* 105 (5), 683-694
- 336 10 Kume, K. *et al.* (1999) mCRY1 and mCRY2 are essential components of the negative  
337 limb of the circadian clock feedback loop. *Cell* 98 (2), 193-205
- 338 11 Preitner, N. *et al.* (2002) The orphan nuclear receptor REV-ERB $\alpha$  controls  
339 circadian transcription within the positive limb of the mammalian circadian oscillator.  
340 *Cell* 110 (2), 251-260
- 341 12 Zhang, E.E. *et al.* (2009) A genome-wide RNAi screen for modifiers of the circadian  
342 clock in human cells. *Cell* 139 (1), 199-210
- 343 13 Robles, M.S. *et al.* (2010) Identification of RACK1 and protein kinase Calpha as  
344 integral components of the mammalian circadian clock. *Science* 327 (5964), 463-466
- 345 14 Hardin, P.E. *et al.* (1990) Feedback of the *Drosophila* period gene product on  
346 circadian cycling of its messenger RNA levels. *Nature* 343 (6258), 536-540
- 347 15 Dunlap, J.C. and Loros, J.J. (2006) How fungi keep time: circadian system in  
348 *Neurospora* and other fungi. *Curr Opin Microbiol* 9 (6), 579-587
- 349 16 Hardin, P.E. (2005) The circadian timekeeping system of *Drosophila*. *Curr Biol* 15  
350 (17), R714-722
- 351 17 Ko, C.H. and Takahashi, J.S. (2006) Molecular components of the mammalian  
352 circadian clock. *Hum Mol Genet* 15 Spec No 2, R271-277
- 353 18 Giudicelli, F. *et al.* (2007) Setting the tempo in development: an investigation of the  
354 zebrafish somite clock mechanism. *PLoS Biol* 5 (6), e150
- 355 19 Mehra, A. *et al.* (2009) Post-translational modifications in circadian rhythms. *Trends*  
356 *Biochem Sci* 34 (10), 483-490
- 357 20 Lee, H. *et al.* (2009) Essential roles of CKIdelta and CKIepsilon in the mammalian  
358 circadian clock. *Proc Natl Acad Sci U S A* 106 (50), 21359-21364
- 359 21 Hirata, H. *et al.* (2004) Instability of Hes7 protein is crucial for the somite  
360 segmentation clock. *Nat Genet* 36 (7), 750-754

- 361 22 Filipowicz, W. *et al.* (2008) Mechanisms of post-transcriptional regulation by  
362 microRNAs: are the answers in sight? *Nat Rev Genet* 9 (2), 102-114
- 363 23 Glisovic, T. *et al.* (2008) RNA-binding proteins and post-transcriptional gene  
364 regulation. *FEBS Lett* 582 (14), 1977-1986
- 365 24 Keene, J.D. (2010) Minireview: global regulation and dynamics of ribonucleic Acid.  
366 *Endocrinology* 151 (4), 1391-1397
- 367 25 So, W.V. and Rosbash, M. (1997) Post-transcriptional regulation contributes to  
368 *Drosophila* clock gene mRNA cycling. *Embo J* 16 (23), 7146-7155
- 369 26 Woo, K.C. *et al.* (2009) Mouse period 2 mRNA circadian oscillation is modulated by  
370 PTB-mediated rhythmic mRNA degradation. *Nucleic Acids Res* 37 (1), 26-37
- 371 27 Woo, K.C. *et al.* (2010) Circadian amplitude of cryptochrome 1 is modulated by  
372 mRNA stability regulation via cytoplasmic hnRNP D oscillation. *Mol Cell Biol* 30 (1),  
373 197-205
- 374 28 Kojima, S. *et al.* (2007) LARK activates posttranscriptional expression of an essential  
375 mammalian clock protein, PERIOD1. *Proc Natl Acad Sci U S A* 104 (6), 1859-1864
- 376 29 Huang, Y. *et al.* (2009) Altered LARK expression perturbs development and  
377 physiology of the *Drosophila* PDF clock neurons. *Mol Cell Neurosci* 41 (2), 196-205
- 378 30 Bartel, D.P. (2009) MicroRNAs: target recognition and regulatory functions. *Cell* 136  
379 (2), 215-233
- 380 31 Cheng, H.Y. *et al.* (2007) microRNA modulation of circadian-clock period and  
381 entrainment. *Neuron* 54 (5), 813-829
- 382 32 Kim, T.D. *et al.* (2005) Rhythmic serotonin N-acetyltransferase mRNA degradation is  
383 essential for the maintenance of its circadian oscillation. *Mol Cell Biol* 25 (8), 3232-  
384 3246
- 385 33 Kim, T.D. *et al.* (2007) Rhythmic control of AANAT translation by hnRNP Q in  
386 circadian melatonin production. *Genes Dev* 21 (7), 797-810
- 387 34 Kornmann, B. *et al.* (2007) System-driven and oscillator-dependent circadian  
388 transcription in mice with a conditionally active liver clock. *PLoS Biol* 5 (2), e34
- 389 35 Hughes, M.E. *et al.* (2009) Harmonics of circadian gene transcription in mammals.  
390 *PLoS Genet* 5 (4), e1000442
- 391 36 Lidder, P. *et al.* (2005) Circadian control of messenger RNA stability. Association  
392 with a sequence-specific messenger RNA decay pathway. *Plant Physiol* 138 (4), 2374-  
393 2385
- 394 37 Sire, C. *et al.* (2009) Diurnal oscillation in the accumulation of *Arabidopsis*  
395 microRNAs, miR167, miR168, miR171 and miR398. *FEBS Lett* 583 (6), 1039-1044
- 396 38 Yang, M. *et al.* (2008) Circadian regulation of a limited set of conserved microRNAs  
397 in *Drosophila*. *BMC Genomics* 9, 83
- 398 39 Xu, S. *et al.* (2007) MicroRNA (miRNA) transcriptome of mouse retina and  
399 identification of a sensory organ-specific miRNA cluster. *J Biol Chem* 282 (34),  
400 25053-25066
- 401 40 Mittag, M. *et al.* (1994) Circadian expression of the luciferin-binding protein  
402 correlates with the binding of a protein to the 3' untranslated region of its mRNA. *Proc*  
403 *Natl Acad Sci U S A* 91 (12), 5257-5261
- 404 41 Iliev, D. *et al.* (2006) A heteromeric RNA-binding protein is involved in maintaining  
405 acrophase and period of the circadian clock. *Plant Physiol* 142 (2), 797-806
- 406 42 Voytsekh, O. *et al.* (2008) Both subunits of the circadian RNA-binding protein  
407 CHLAMY1 can integrate temperature information. *Plant Physiol* 147 (4), 2179-2193
- 408 43 Kim, J.S. *et al.* (2009) Muscleblind-like 2: circadian expression in the mammalian  
409 pineal gland is controlled by an adrenergic-cAMP mechanism. *J Neurochem* 110 (2),  
410 756-764
- 411 44 Baggs, J. and Green, C. (2003) Nocturnin, a Deadenyase in *Xenopus laevis* Retina. A  
412 Mechanism for Posttranscriptional Control of Circadian-Related mRNA. *Curr Biol* 13  
413 (3), 189-198

414 45 Green, C.B. *et al.* (2007) Loss of Nocturnin, a circadian deadenylase, confers  
415 resistance to hepatic steatosis and diet-induced obesity. *Proc Natl Acad Sci U S A* 104  
416 (23), 9888-9893

417 46 Reddy, A.B. *et al.* (2006) Circadian orchestration of the hepatic proteome. *Curr Biol*  
418 16 (11), 1107-1115

419 47 Houseley, J. *et al.* (2006) RNA-quality control by the exosome. *Nat Rev Mol Cell Biol*  
420 7 (7), 529-539

421 48 Guo, J. *et al.* (2009) The exosome regulates circadian gene expression in a  
422 posttranscriptional negative feedback loop. *Cell* 138 (6), 1236-1246

423 49 Heintzen, C. *et al.* (1997) AtGRP7, a nuclear RNA-binding protein as a component of  
424 a circadian-regulated negative feedback loop in *Arabidopsis thaliana*. *Proc Natl Acad*  
425 *Sci U S A* 94 (16), 8515-8520

426 50 Schoning, J.C. *et al.* (2007) Auto-regulation of the circadian slave oscillator  
427 component AtGRP7 and regulation of its targets is impaired by a single RNA  
428 recognition motif point mutation. *Plant J* 52 (6), 1119-1130

429 51 Schoning, J.C. *et al.* (2008) Reciprocal regulation of glycine-rich RNA-binding  
430 proteins via an interlocked feedback loop coupling alternative splicing to nonsense-  
431 mediated decay in *Arabidopsis*. *Nucleic Acids Res* 36 (22), 6977-6987

432 52 Wollerton, M.C. *et al.* (2004) Autoregulation of polypyrimidine tract binding protein  
433 by alternative splicing leading to nonsense-mediated decay. *Mol Cell* 13 (1), 91-100

434 53 Rahman, L. *et al.* (2002) Alternative splicing of brain-specific PTB defines a tissue-  
435 specific isoform pattern that predicts distinct functional roles. *Genomics* 80 (3), 245-  
436 249

437 54 Spellman, R. *et al.* (2007) Crossregulation and functional redundancy between the  
438 splicing regulator PTB and its paralogs nPTB and ROD1. *Mol Cell* 27 (3), 420-434

439 55 Boutz, P.L. *et al.* (2007) A post-transcriptional regulatory switch in polypyrimidine  
440 tract-binding proteins reprograms alternative splicing in developing neurons. *Genes*  
441 *Dev* 21 (13), 1636-1652

442 56 Dembowski, J.A. and Grabowski, P.J. (2009) The CUGBP2 splicing factor regulates  
443 an ensemble of branchpoints from perimeter binding sites with implications for  
444 autoregulation. *PLoS Genet* 5 (8), e1000595

445 57 Dequeant, M.L. *et al.* (2006) A complex oscillating network of signaling genes  
446 underlies the mouse segmentation clock. *Science* 314 (5805), 1595-1598

447 58 Morales, A. *et al.* (2002) Periodic Lunatic fringe expression is controlled during  
448 segmentation by a cyclic transcriptional enhancer responsive to notch signaling. *Dev*  
449 *Cell* 3, 63-74

450 59 Gajewski, M. *et al.* (2003) Anterior and posterior waves of cyclic her1 gene  
451 expression are differentially regulated in the presomitic mesoderm of zebrafish.  
452 *Development* 130 (18), 4269-4278

453 60 Davis, R. *et al.* (2001) Molecular targets of vertebrate segmentation: two mechanisms  
454 control segmental expression of *Xenopus* Hairy2 during somite formation. *Dev Cell* 1,  
455 553-565

456 61 Hitachi, K. *et al.* (2008) Tbx6, Thylacine1, and E47 synergistically activate bowline  
457 expression in *Xenopus* somitogenesis. *Dev Biol* 313 (2), 816-828

458 62 Hilgers, V. *et al.* (2005) In vivo analysis of mRNA stability using the Tet-Off system  
459 in the chicken embryo. *Dev Biol* 284 (2), 292-300

460 63 Lewis, J. (2003) Autoinhibition with transcriptional delay: a simple mechanism for the  
461 zebrafish somitogenesis oscillator. *Curr Biol* 13 (16), 1398-1408

462 64 Dill, K.K. and Amacher, S.L. (2005) *tortuga* refines Notch pathway gene expression in  
463 the zebrafish presomitic mesoderm at the post-transcriptional level. *Dev Biol* 287 (2),  
464 225-236



- 465 65 Gautier-Courteille, C. *et al.* (2004) EDEN-BP-dependent post-transcriptional  
466 regulation of gene expression in *Xenopus* somitic segmentation. *Development* 131  
467 (24), 6107-6117
- 468 66 Huot, M.E. *et al.* (2005) The RNA-binding protein fragile X-related 1 regulates somite  
469 formation in *Xenopus laevis*. *Mol Biol Cell* 16 (9), 4350-4361
- 470 67 Cibois, M. *et al.* (2010) A strategy to analyze the phenotypic consequences of  
471 inhibiting the association of an RNA-binding protein with a specific RNA. *Rna* 16 (1),  
472 10-15
- 473 68 Nagel, R. *et al.* (2009) The miRNA-192/194 cluster regulates the Period gene family  
474 and the circadian clock. *Febs J* 276 (19), 5447-5455
- 475 69 Gatfield, D. *et al.* (2009) Integration of microRNA miR-122 in hepatic circadian gene  
476 expression. *Genes Dev* 23 (11), 1313-1326
- 477 70 Kadener, S. *et al.* (2009) A role for microRNAs in the *Drosophila* circadian clock.  
478 *Genes Dev* 23 (18), 2179-2191
- 479 71 Kwak, E. *et al.* (2006) Essential role of 3'-untranslated region-mediated mRNA decay  
480 in circadian oscillations of mouse Period3 mRNA. *J Biol Chem* 281 (28), 19100-  
481 19106
- 482 72 Wilsbacher, L.D. *et al.* (2002) Photic and circadian expression of luciferase in  
483 mPeriod1-luc transgenic mice *in vivo*. *Proc Natl Acad Sci U S A* 99 (1), 489-494
- 484 73 Wang, D.O. *et al.* (2010) Spatially restricting gene expression by local translation at  
485 synapses. *Trends Neurosci* 33 (4), 173-182
- 486 74 Giampietro, P.F. *et al.* (2009) Progress in the understanding of the genetic etiology of  
487 vertebral segmentation disorders in humans. *Ann N Y Acad Sci* 1151, 38-67
- 488 75 Gould, E.L. *et al.* (2000) Melatonin profiles and sleep characteristics in boys with  
489 fragile X syndrome: a preliminary study. *Am J Med Genet* 95 (4), 307-315
- 490 76 Zhang, J. *et al.* (2008) Fragile X-related proteins regulate mammalian circadian  
491 behavioral rhythms. *Am J Hum Genet* 83 (1), 43-52
- 492 77 Ule, J. *et al.* (2003) CLIP identifies Nova-regulated RNA networks in the brain.  
493 *Science* 302 (5648), 1212-1215
- 494 78 Licatalosi, D.D. *et al.* (2008) HITS-CLIP yields genome-wide insights into brain  
495 alternative RNA processing. *Nature* 456 (7221), 464-469
- 496 79 Sanford, J.R. *et al.* (2009) Splicing factor SFRS1 recognizes a functionally diverse  
497 landscape of RNA transcripts. *Genome Res* 19 (3), 381-394
- 498 80 Yeo, G.W. *et al.* (2009) An RNA code for the FOX2 splicing regulator revealed by  
499 mapping RNA-protein interactions in stem cells. *Nat Struct Mol Biol* 16 (2), 130-137
- 500 81 Chi, S.W. *et al.* (2009) Argonaute HITS-CLIP decodes microRNA-mRNA interaction  
501 maps. *Nature* 460 (7254), 479-486
- 502 82 Zisoulis, D.G. *et al.* (2010) Comprehensive discovery of endogenous Argonaute  
503 binding sites in *Caenorhabditis elegans*. *Nat Struct Mol Biol* 17 (2), 173-179
- 504 83 Robertson, J.B. *et al.* (2008) Real-time luminescence monitoring of cell-cycle and  
505 respiratory oscillations in yeast. *Proc Natl Acad Sci U S A* 105 (46), 17988-17993
- 506 84 Veldhuis, J.D. *et al.* (1986) Spectrum of the pulsatile characteristics of LH release in  
507 normal men. *J Androl* 7 (2), 83-92
- 508 85 Knight, J.E. *et al.* (2000) mRNA stability and polysome loss in hibernating Arctic  
509 ground squirrels (*Spermophilus parryi*). *Mol Cell Biol* 20 (17), 6374-6379.
- 510 86 Hoppensteadt, F.C. and Keller, J.B. (1976) Synchronization of periodical cicada  
511 emergences. *Science* 194 (4262), 335-337
- 512 87 Kawamura, A. *et al.* (2005) Zebrafish hairy/enhancer of split protein links FGF  
513 signaling to cyclic gene expression in the periodic segmentation of somites. *Genes*  
514 *Dev* 19 (10), 1156-1161
- 515 88 Kuersten, S. and Goodwin, E.B. (2003) The power of the 3' UTR: translational control  
516 and development. *Nat Rev Genet* 4 (8), 626-637

- 517 89 Mauxion, F. *et al.* (2009) BTG/TOB factors impact deadenylases. *Trends Biochem Sci*  
518 34 (12), 640-647
- 519 90 Garceau, N.Y. *et al.* (1997) Alternative initiation of translation and time-specific  
520 phosphorylation yield multiple forms of the essential clock protein FREQUENCY.  
521 *Cell* 89 (3), 469-476
- 522 91 Le Hir, H. and Seraphin, B. (2008) EJC's at the heart of translational control. *Cell* 133  
523 (2), 213-216
- 524 92 Kamath, R. *et al.* (2003) Systematic functional analysis of the *Caenorhabditis elegans*  
525 genome using RNAi. *Nature* 421, 231-237
- 526 93 Dietzl, G. *et al.* (2007) A genome-wide transgenic RNAi library for conditional gene  
527 inactivation in *Drosophila*. *Nature* 448 (7150), 151-156

528 **Glossary box**

529 **3' Untranslated Region (3'UTR):** Region of the mRNA 3' to the translation stop codon.

530 **Alternative splicing:** Various ways to skip introns and splice exons. This mechanism  
531 generates a large diversity of mRNA molecules from a single gene. Alternative splicing  
532 includes mutually exclusive exons (where splicing leads to the inclusion of either of two  
533 exons), exon skipping, intron retention, alternative 5' or 3' splice sites (leading to the retention  
534 of all or only part of an exon) and alternative terminal exons.

535 **Circadian rhythm:** A cycle one day long (Latin *circa*, about, and *dies*, day). The period of a  
536 circadian rhythm is 24 h when the organism is grown under a light-dark cycle (12h light, 12h  
537 darkness), and about 24 h when the organism is released into free-running condition. Several  
538 parameters cycle in circadian rhythms, the most obvious one in mammals being sleep and  
539 wake.

540 **Free-running rhythm:** Circadian rhythm in the absence of external cues (like constant  
541 darkness and temperature).

542 **Half-life.** Time required in the absence of synthesis to achieve degradation of half the initial  
543 amount of a molecule (like an mRNA).

544 **Infradian rhythm:** A cycle of length above 24h.

545 **Melatonin:** Circulating hormone secreted by the pineal gland during the night in mammals. It  
546 relays the circadian rhythm imposed by the central nervous system to the peripheral organs.

547 **miRNA (micro RNA):** Short double-stranded RNA, encoded by the genome, that controls  
548 gene expression at several levels. In vertebrates, a prevalent feature of miRNAs is their  
549 capacity to specifically repress the translation of target mRNAs by (limited) sequence  
550 complementarity.

551 **Period:** Time interval between two reference points (two peaks for example). Inverse of  
552 frequency.

553 **Presomitic mesoderm:** Posterior, non-segmented mesoderm, in which the segmentation  
554 clock is active and from which segmented somites periodically bud off.

555 **Somites:** Transient embryonic repeated mesodermal structures. They are the origin of adult  
556 skeletal muscles, bones and derm.

557 **Somitic segmentation:** Organisation of the somites as repeated units along the embryonic  
558 antero-posterior axis.

559 **Suprachiasmatic nucleus (SCN):** A region of the hypothalamus. The master circadian clock  
560 is located within the SCN.

561 **Ultradian rhythm:** A cycle of length shorter than 24h (e.g. the segmentation clock).

562 **Box 1. Some examples of biological rhythms**

563

564 Depending on the period, biorhythms are classified as ultradian (period  $T < 24\text{h}$ ), infradian  
565 ( $T > 24\text{h}$ ) and circadian ( $T \sim 24\text{h}$ ). Ultradian rhythms include heart beating ( $T =$ fractions of  
566 seconds to seconds), sleep episodes ( $T =$ tens of minutes), respiratory oscillations in yeasts  
567 ( $T = 1\text{--}5\text{h}$  [83]), somitic segmentation in vertebrates ( $T = 30$  minutes in Zebrafish, 2h in mice  
568 [2]), or pulses of LH secretion by the pituitary gland ( $T \sim 3\text{h}$  in men [84]). Infradian rhythms  
569 include successions of torpor and arousal during the hibernation of small mammals  
570 ( $T =$ several days [85]), female menstrual cycles ( $T =$ several days to several months), annual  
571 rhythms (flowering of most plants), and even pluri-annual rhythms such as the emergence of  
572 Cicada [86].

573 **Box 2. The vertebrate segmentation clock.**

574 Please refer to the accompanying figure.

575 Title of the figure "The zebrafish core segmentation clock"

576

577 In zebrafish, the core segmentation clock consists of Her1 and Her7 proteins (see Figure).

578 Homodimers or heterodimers of these proteins bind to their own promoters and repress their

579 transcription. Taking into account transcriptional and translational delays, this results in

580 oscillating levels of these proteins. Furthermore, Her1/7 duplexes repress the transcription of

581 Delta-C, a transmembrane Notch ligand. When bound by its ligand, the Notch transmembrane

582 receptor undergoes a limited proteolysis that releases the Notch intracellular domain (NICD)

583 in the cytoplasm. NICD is then translocated to the nucleus. Together with Su(H) protein, the

584 NICD stimulates the transcription of target genes including *Her1* and *Her7*. The stimulation

585 of *Her1/7* transcription by Delta-C expressed in adjacent cells, and the ensuing repression of

586 *Delta-C* gene by Her1/7 achieves coordinated oscillations in neighbouring cells [18,63].

587 Her13.2 reinforces the transcriptional inhibition mediated by Her1/7, and it is controlled by

588 the FGF pathway. This links the Notch and FGF signalling pathways [87]. Several other

589 genes are downstream of Her1/7 and are involved in somitic segmentation. In amniotes

590 (chick, mouse), the segmentation clock is more complex. It requires oscillations of the Notch

591 modulator Lunatic fringe, and of tens of mRNAs that encode proteins belonging to the FGF

592 and Wnt signalling pathways in addition to Notch [57].

593 **Box 3. Different levels of posttranscriptional controls of gene expression.**

594

595 Please refer to the accompanying figure.

596 Title of the figure "pre-mRNA and mRNA fate in eukaryotic cells"

597

598 The posttranscriptional controls are exerted on RNA molecules and are indicated in red on the  
599 figure. Concomitantly with nuclear transcription, pre-mRNAs are matured to mRNAs. Pre-  
600 mRNA maturation refers to three events: 5' capping, 3' cleavage and polyadenylation, and  
601 intron excision coupled with exon splicing. Most pre-mRNAs can be cleaved and  
602 polyadenylated at several sites (alternative cleavage/polyadenylation) and/or undergo several  
603 splicing patterns (alternative splicing. In the figure, the second exon is either skipped or  
604 spliced). Due to alternative cleavage/polyadenylation and alternative splicing, a large variety  
605 of mRNAs can be obtained from a given pre-mRNA.

606 After nucleo-cytoplasmic export, mRNA translation and decay are controlled, and the 3'  
607 poly(A) tail is a major site for these controls. Polyadenylated mRNAs are much more actively  
608 translated than deadenylated mRNAs. The initiation factor eIF4G, that recruits the small  
609 ribosomal subunit, is able to interact simultaneously with the 5' cap-binding protein eIF4E and  
610 the 3' Poly(A) binding protein. The connection between mRNA 5' (cap) and 3' (poly(A) tail)  
611 ends strongly stimulates translation [88]. In addition, polyadenylated mRNAs are much more  
612 stable than deadenylated mRNAs. For most mRNAs, deadenylation is the rate-limiting step of  
613 mRNA decay, and several factors that control mRNA stability do so by regulating the  
614 deadenylation rate. In higher eukaryotes, the major pathway for mRNA decay is poly(A) tail  
615 removal (deadenylation) followed by RNA exosome-mediated 3' to 5' exonucleolytic  
616 degradation. [89]. The 5'-most AUG codon is generally the translation initiation codon, but  
617 more distal initiation codons can also be used (alternative initiation of translation), resulting in  
618 alternative protein isoforms. This mechanism was described for instance for the mRNA that  
619 encodes FRQ, a component of the *N. Crassa* circadian clock [90].

620 Nuclear and cytoplasmic controls are tightly coupled. A complex (EJC, exon junction  
621 complex) is assembled during splicing immediately upstream of exon junctions, and remains  
622 associated with the mRNA during nucleocytoplasmic export. This hallmark of a nuclear event  
623 then influences cytoplasmic mRNA translation and degradation [91]. For example, the EJC is  
624 involved in the recognition and rapid degradation of mRNAs containing a premature stop  
625 codon by the ‘nonsense-mediated mRNA decay’ (NMD) pathway [91]. In addition,  
626 alternative splicing can lead to mature transcripts that contain alternative 3' untranslated  
627 regions (3'UTR), that are instrumental in mRNA stability and translation [88]. Consequently,  
628 alternative cleavage/polyadenylation or splicing impacts mRNA half-life or translation.



629 **Box 4. Future questions**

630 - Uniform mRNA instability is the only mode of posttranscriptional controls demonstrated in  
631 the segmentation clock. Do oscillating mRNA stability and/or oscillating mRNA translation  
632 also play a role?

633 - In the circadian clock, the described mechanisms relate to most posttranscriptional controls  
634 found to be governing the expression of other non-clock-related gene programs, but mRNA  
635 intracellular traffic and local translation were not reported. Since they are prevalent  
636 mechanisms in neurons [73], one could ask if they have a function in the circadian clock.

637 - A posttranscriptional feedback loop was demonstrated in *N. crassa* circadian clock [48], and  
638 the levels of some RNA-BPs oscillate in mammalian circadian clocks [28,33]. Are there  
639 posttranscriptional feedback loops in vertebrate clocks that could account for the oscillations  
640 of these RNA-BPs?

641 - Systematic gene inactivations were reported in lower metazoans [92,93], and several genes  
642 were disrupted by homologous recombination in mice. Some of them encode RNA-BPs or  
643 miRNAs. Which inactivations lead to clock troubles, demonstrating an involvement of the  
644 corresponding gene products in clock setting or robustness?

645 - What are the posttranscriptional networks in clocks? For the RNA-BPs and the miRNAs that  
646 are involved in clocks, what are the associated mRNAs?

647 - Are deregulations of posttranscriptional networks in clocks at the origin of human diseases?

648 **Figure legends**

649 **Figure 1. The mammalian circadian clock and its three layers of control**

650 **(a)** Master circadian pacemaker in the suprachiasmatic nucleus (SCN). The Clock–Bmal1  
651 complex directly stimulates the transcription of *Per*, *Cry*, *Rev-Erb $\alpha$* , and of output clock-  
652 controlled genes (CCGs) via binding to the E-box. Oscillatory activity of the Clock–Bmal1  
653 complex is achieved by two negative feedback loops: the Per–Cry complex inhibits Clock–  
654 Bmal1, and *Bmal1* transcription is repressed by binding of Rev-Erb $\alpha$  to the RRE (ROR  
655 response element). **(b)** Relationships between transcriptional, posttranscriptional and  
656 posttranslational layers in the control of *Per* genes expression. Since Per proteins contribute to  
657 the control of the Clock–Bmal1 complex, fine-tuning their levels is required to obtain  
658 oscillations of clock genes. The levels of Per proteins are regulated at a transcriptional level  
659 (yellow layer) by the Clock–Bmal1 complex (see Figure 1a). They are regulated at a  
660 posttranslational level too (green layer), among others as Casein-kinase1- $\delta$  and - $\epsilon$  mediate Per  
661 phosphorylation that targets them to ubiquitin/proteasome degradation [19,20]. Recent results  
662 demonstrate that a third layer (posttranscriptional controls, red) should be added to complete  
663 the picture. The oscillating controls (transcription, mRNA translation and degradation) are in  
664 capital letters.

665

666 **Figure 2. Posttranscriptional controls exerted on mRNAs encoding proteins involved in**  
667 **circadian rhythms**

668 Arrows and blunt-end lines towards ribosomes (brown) indicate stimulation and inhibition,  
669 respectively, of mRNA translation. Arrows towards the exonucleolytic enzyme (yellow)  
670 indicate stimulation of mRNA decay. The sinusoidal symbols on the right of the factors  
671 involved in posttranscriptional controls indicate oscillating levels of these factors. **(a)**  
672 Components of the master circadian clock in the SCN. **(b)** Aanat, a pineal, rate-limiting  
673 enzyme in melatonin synthesis.

<b>Gene</b>	<b>Function in the clock</b>	<b>Evidence for posttranscriptional controls</b>	<b>References</b>
<i>Llnfg</i> in amniotes	Encodes modulator of Notch signalling	mRNA instability inferred from expression pattern in mice; 3'UTR of chick mRNA confers rapid degradation to a reporter mRNA	[58,62]
zebrafish <i>Her1</i>	Encodes component of the core clock	The expression pattern of a reporter mRNA controlled by <i>Her1</i> promoter is different from that of endogenous <i>Her1</i> due to increased mRNA stability. Expression pattern in the <i>Tortuga</i> mutant consistent with <i>Tortuga</i> gene product being responsible for <i>Her1</i> mRNA instability	[59,64]
<i>Xenopus Hairy 2a, Hairy 1, Esr5, Nrarp, Bowline, Chick Hairy 1, Mouse Hes1, human HES4</i>	Mouse Hes1 and human HES4 may be components of segmentation clock. The other genes encode factors downstream of the segmentation clock. Some of them are involved in setting the antero-posterior polarity of forming somites	In <i>Xenopus</i> , the 3'UTR of <i>Hairy 2a</i> confers instability on a reporter mRNA. The expression pattern of <i>Hairy 2a</i> or <i>Bowline</i> was recapitulated in transgenic embryos with the appropriate promoter and a 3'UTR of one of these genes, but not with a 3'UTR of a stable mRNA.	[60,61]
<i>Xenopus Su(H)</i> (homologue of mammalian <i>Rbpj</i> )	Binds to Notch intracellular domain to stimulate expression of Notch target genes	mRNA instability is conferred by association with the RNA-BP Celf1. A specific impairment of the interaction between Celf1 and <i>Su(H)</i> mRNA causes segmentation defects.	[65,67]

674

675 **Table 1. Posttranscriptional controls of gene expression in the segmentation clock.**

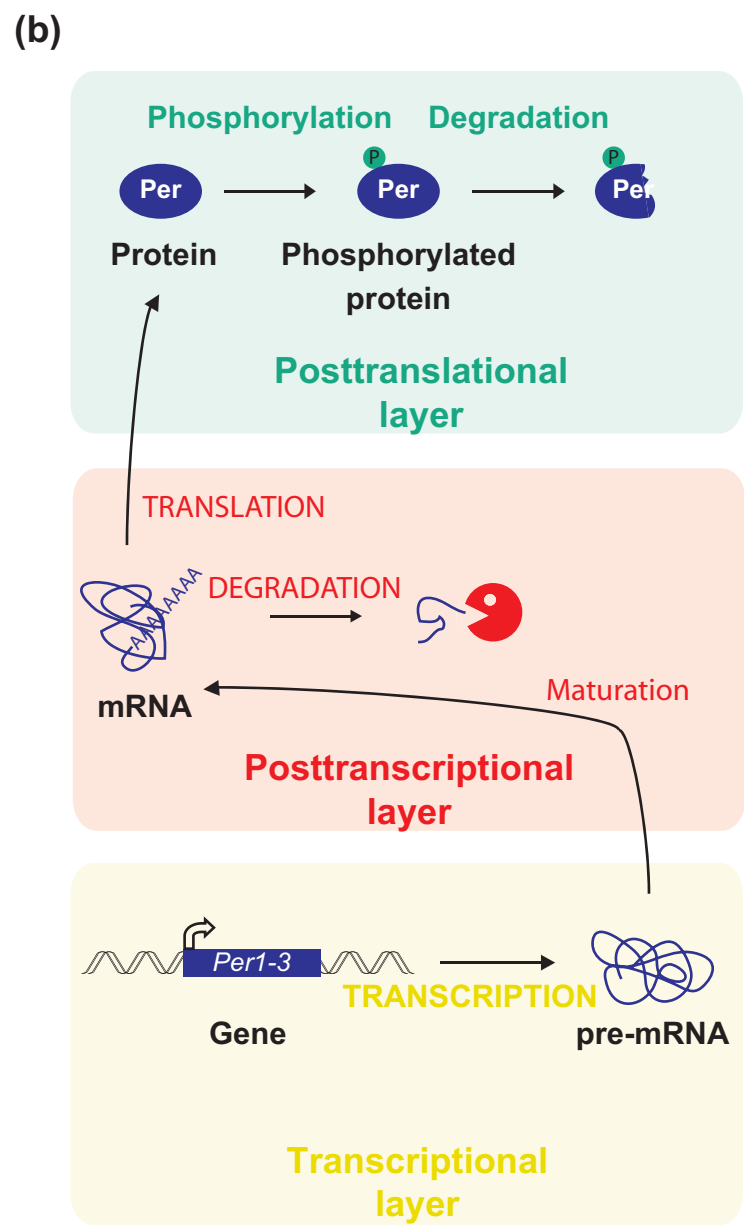
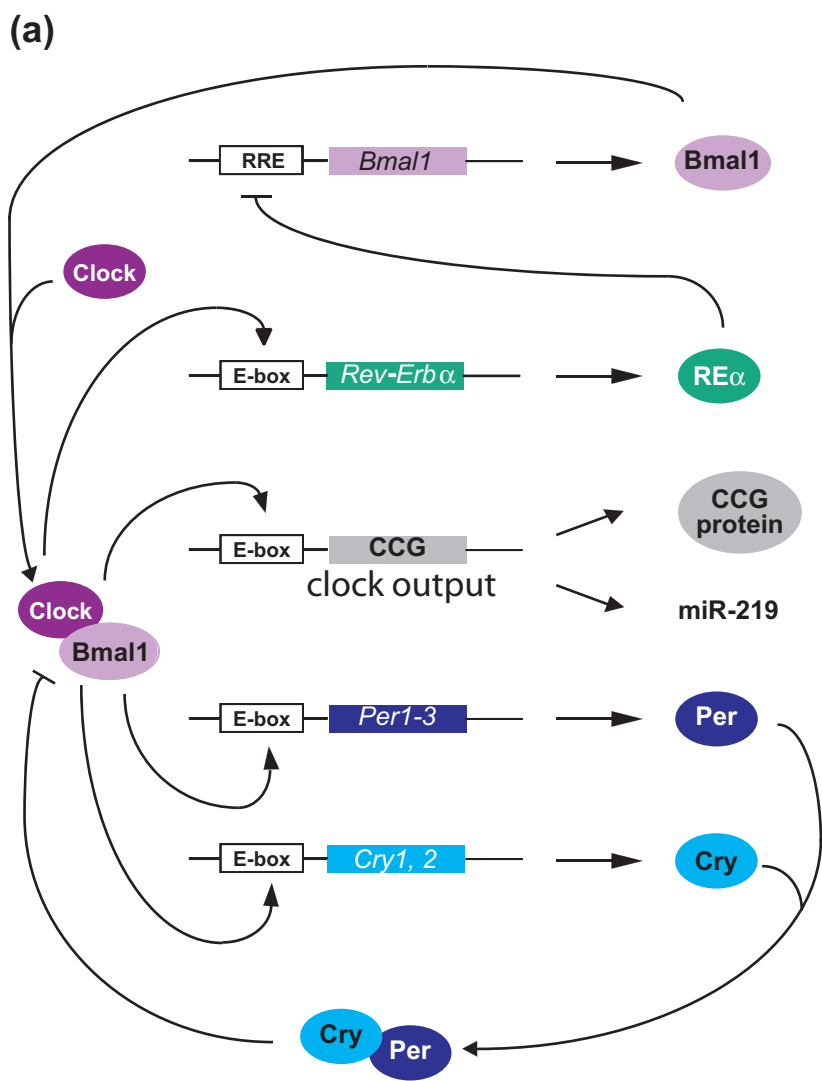
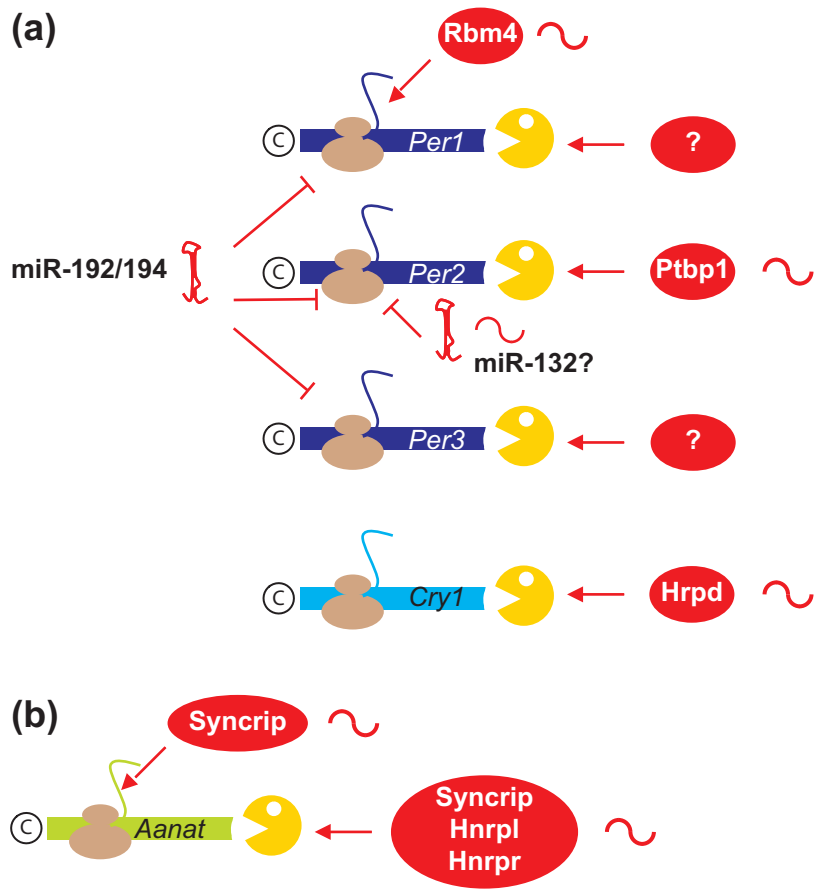
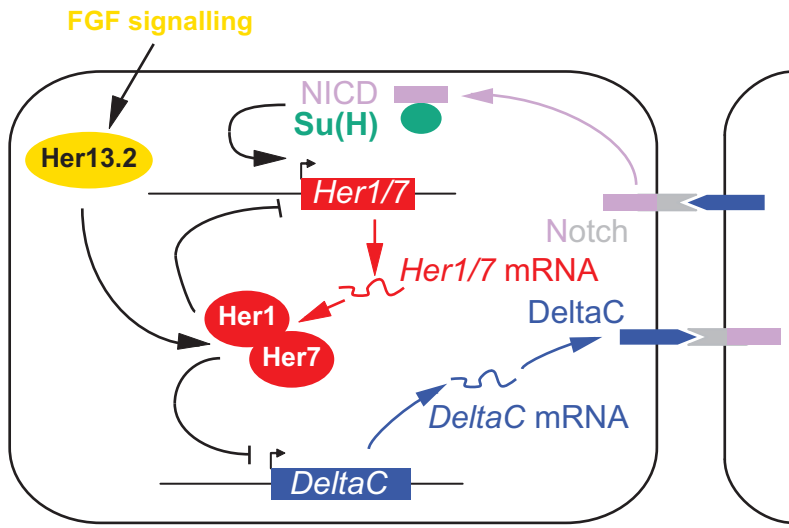


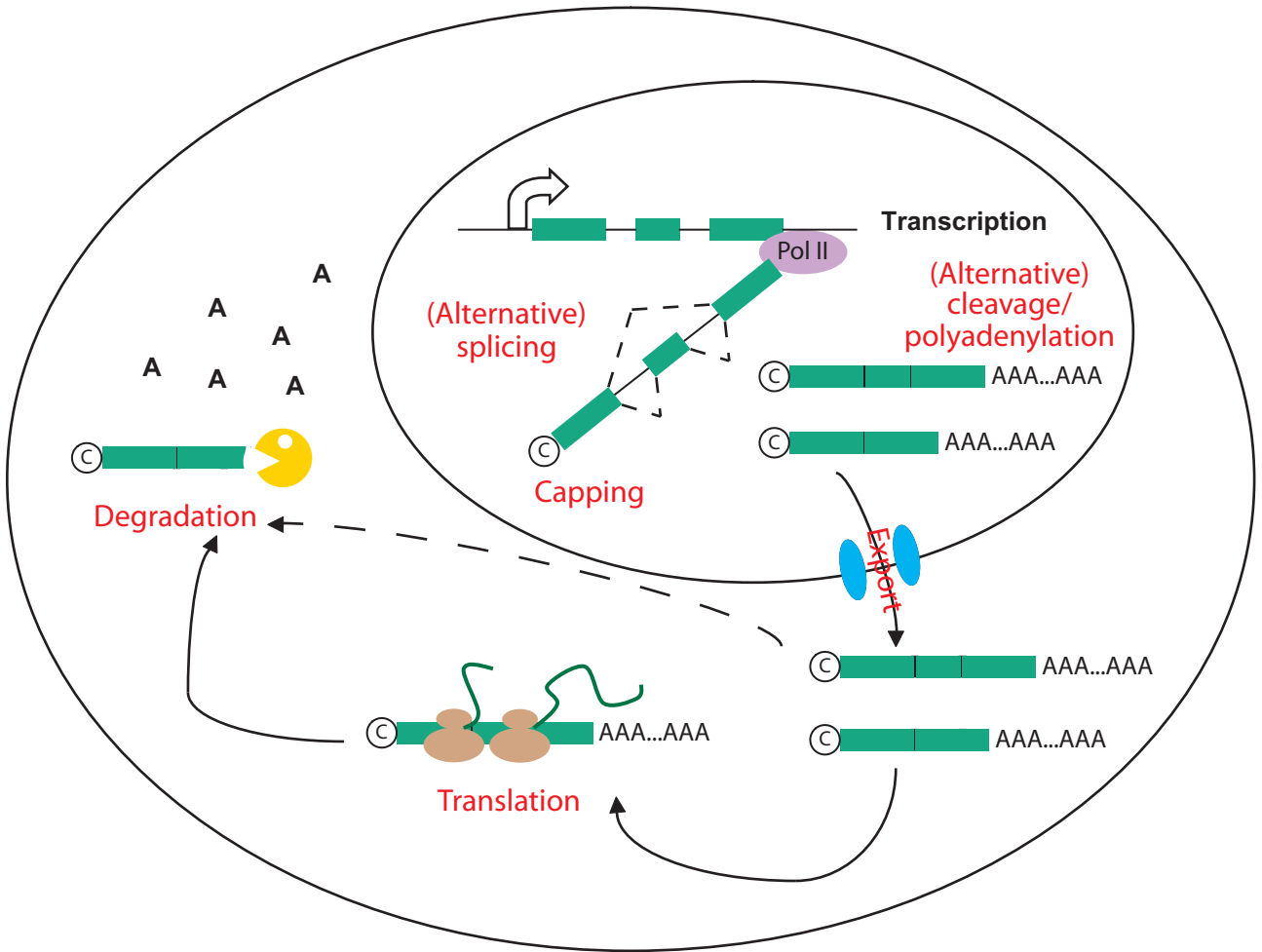
FIGURE 1



**FIGURE 2**



**FIGURE OF BOX 2**



**FIGURE OF BOX 3**