Minireview

Circadian clocks and natural antisense RNA

Susan K. Crosthwaite*

School of Biological Sciences, Smith Building, University of Manchester, Oxford Road, Manchester M13 9PT, UK

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Abstract Eukaryotes regulate gene expression in a number of different ways. On a daily and seasonal timescale, the orchestration of gene expression is to a large extent governed by circadian clocks. These endogenous timekeepers enable organisms to prepare for predictable environmental conditions from one day to the next and thus allow adaptation to a given temporal niche. In general, circadian clocks have been shown to employ the classical transcriptional and posttranscriptional control mechanisms to generate rhythmicity. However, the discovery of antisense clock gene transcripts suggests that mechanisms of gene regulation operating through antisense RNA may also be integral to the circadian clockwork. Following a brief history of the impact of genetic and molecular techniques in aiding our understanding of circadian clocks, this review concentrates on the few examples of antisense clock gene transcripts so far investigated and their effect on circadian timing.

Keywords: Circadian; Clock; RNA; Non-coding; Antisense; *Neurospora*

1. Historical background

Organisms exhibit behaviour with 24-h periodicity that in many cases is driven by cyclical environmental stimuli. Numerous activities, however, are expressed circadianly (with a periodicity of about -circa, a day- dies) in constant conditions, uncovering a further layer of temporal regulation, which is controlled by endogenous timekeepers [1,2]. Processes regulated by circadian clocks include: the onset of sporulation in the filamentous fungus *Neurospora crassa* [3], the opening of leaves before sunrise and closing before sunset by many plants [4], eclosion of certain insects [5], and the emergence of nocturnal animals before night fall [4]. These rhythms or ''hands'' of the clock are preceded by changes in biochemistry and gene expression. Indeed, in the cyanobacterium, *Synochococcus*, nearly all genes are under clock control [6]. In eukaryotes, transcriptional profiling indicates that roughly 5–10% of genes in fungi [7], plants [8], insects [9] and mammals [10–12] are under clock control. However, only subsets of these genes play a role in behaviour and development. For example, in the liver, some of the key enzymes required for intermediate and xenobiotic metabolism are circadianly regulated [12]. This lends substance to another professed role of circadian clocks which is that they organize gene expression to make best use not only of the external environment but also of the internal environment of the cell, and coordinate appropriate activity in tissues and organs to specific times of day [13]. Intuitively, it has been argued that possession of a circadian clock should increase the fitness of an organism by allowing it to predict and make ready to exploit cyclical changes in the environment. That this is the case is clear from the results of competition studies between long and short period mutants of cyanobacteria; those bacteria whose endogenous clocks have a periodicity closest to the experimental periodicity of the light–dark cycle do indeed have a selective advantage [14].

Circadian clocks not only allow organisms to adjust their schedules to local time on a day-to-day basis, they also feed information on photoperiod into seasonally controlled processes, for example reproductive activity and hibernation in animals [15] and flowering in plants [16]. In addition, circadian clocks are used by some animals for celestial navigation. When using the position of the stars as a compass, account must be taken of their movement across the sky as the Earth turns on its axis. Using their internal clock to measure time, some organisms, e.g., migratory monarch butterflies [17] and starlings [18], are able to compensate for the movement of the sun across the sky and thus orient themselves correctly over the course of a day using the position of the sun as a guide.

Circadian clocks have several defining characteristics over and above a periodicity of approximately one day that are crucial for the tasks mentioned above. One essential feature is that they are temperature compensated, allowing accurate time measurement within a range of physiological temperatures. Additionally, clocks are entrained to a 24-h periodicity by cyclical environmental stimuli such as light, temperature and food availability. These and other important characteristics of circadian clocks in a range of organisms were first catalogued and investigated by physiologists [4] between 1729 and the 1960s. At this time the aspirations of chronobiologists were to determine the molecular components of the timekeeper and explain the characteristics of clocks at the molecular level. For instance: the basis of temperature compensation, how the clock perceives resetting stimuli and how time information from the clock is relayed and regulates output processes [1].

* Fax: +44-161-275-5082.
E-mail address: susan.k.crosthwaite@man.ac.uk (S.K. Crosthwaite).

Abbreviations: ARNT, aryl hydrocarbon nuclear translocator; SIM, single-minded; SCN, supra chiasmatic nuclei; UTR, untranslated region; nNOS, neuronal nitric oxide synthase

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The application of genetic and molecular genetic techniques has allowed the field to begin to answer these questions [19].

2. Use of genetics and molecular tools to investigate central questions in clock biology

A little under 40 years ago, the first genetic approaches in *Drosophila* and *Neurospora* led to the identification of clock genes. The first successes arose from deliberate efforts to isolate clock mutants by screening for altered periodicity of activity in *Drosophila* and asexual spore development in *Neurospora* (Fig. 1). These screens identified fly and fungal strains displaying long and short periods and strains that were arrhythmic [20,21]. In the 1980s, the introduction of easily followed artificial clock phenotypes allowed genetic screens for clock genes in bacteria and in plants. In the photosynthetic bacterium *Synechococcus*, luciferase was inserted throughout the genome and in constant conditions the light emitted from transgenic bacteria in the presence of the substrate luciferin assayed [6]. The surprising result was that nearly all strains emitted light rhythmically with

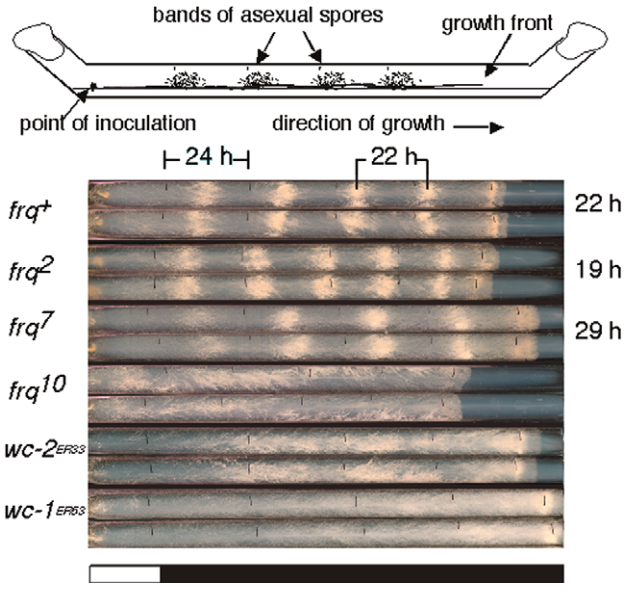

Fig. 1. Monitoring the state of the clock. One of the most easily assayed "hands" of *Neurospora*'s circadian clock is the circadian rhythm of asexual spore (conidia) development. Here, *Neurospora* is inoculated at one end of a race tube (a long glass tube) containing agar growth medium. The cultures are grown in constant light for 24 h and then transferred into constant darkness. This transfer synchronizes the clock to a time corresponding to subjective dusk. Thereafter, a signal is sent from the clock once in every 22 h (the period of the *Neurospora* free-running clock) resulting in a switch from vegetative hyphal growth to asexual spore production. After several days growth "bands" of conidia separated by undifferentiated mycelia are seen along the length of the tube. Since the growth rate is constant, the period and phase of the rhythm can be calculated from the position of these bands relative to one another and to the growth fronts that are marked daily. Top: side view of a race tube showing the circadian development of aerial hyphae. Below: top view of race tubes showing rhythmic conidiation of the frq^{+} and different clock mutant strains: frq^{2}, frq^{7}, frq^{10} (a *frq* null mutant), $wc\text{-}2^{ER33}$ (the WC-2 DNA-binding domain is mutated in this strain) and $wc\text{-}1^{ER53}$ (this strain produces a truncated WC-1 protein lacking a DNA-binding domain). Black vertical lines mark 24-h growth fronts. To the right of the race tubes, the period of each strain is shown. White rectangle indicates growth conditions of 25 °C, constant light; black rectangle indicates 25 °C, constant darkness.

an approximately 24 h period, indicating global control of gene expression by a circadian clock. In *Arabidopsis*, the chlorophyll *a/b* binding protein (*cab*) (one of the first genes shown to be under control of a circadian clock) promoter was fused in front of a luciferase transgene. Transgenic plants were then mutated and in the presence of luciferin, abnormal cycles of gene expression (light-emission) compared to the parental strain indicated possible clock gene mutants [22]. Reverse genetic approaches were also taken. Differential screens for transcripts expressed circadianly in constant conditions through subtractive hybridization revealed components of slave or subordinate oscillators in plants [23] and clock-controlled genes in flies, fungi and mice [19]. Carried out before the advent of gene chips, these screens gave the first indication of the wide range of cellular processes under clock control.

Mutant genes conferring altered periodicity in *Drosophila* and *Neurospora* were mapped and then cloned by functional complementation using rescue of the wild-type periodicity as evidence of the presence of a clock gene. *period* (*per*) *in Drosophila* [24] and *frequency* (*frq*) in *Neurospora* [25] were the first clock genes to be cloned. Both encode proteins that control levels of their own transcripts and are components of negative feedback loops that are instrumental in the generation of circadian rhythmicity [26,27].

In *Neurospora* the first positive elements identified, that are responsible for the activation of clock genes, were encoded by the *white collar* (*wc*) genes [28], two zinc finger transcription factors [29,30] that bind to the promoter of *frq* as a complex and switch on transcription [31,32]. These were closely followed by the discovery of the mouse genes *Clock* and *Bmal* [33] and *dclock* and *dbmal* in *Drosophila* [34,35]: both encode basic helix loop helix transcription factors. In *Neurospora*, the negative feedback of FRQ on the WC proteins results in cycling levels of *frq* RNA and protein. Lowest levels of *frq* RNA occur during the subjective night and peak during the subjective morning [27,31] (Fig. 2A). Cycling levels of FRQ protein follow with a 6–8 h delay. FRQ enters the nucleus soon after translation [36] where it blocks the action of the positive WHITE COLLAR COMPLEX (WCC) [31], thus preventing further accumulation of *frq* transcript. Similar processes using different proteins occur in *Drosophila* and mammals [37].

Recently, feed-forward loops have been revealed. In *Neurospora*, FRQ not only represses but also promotes increased levels of *wc-2* [38] and translation of WHITE-COLLAR-1 (WC-1) [39]. Thus, when FRQ is high it represses transcription of *frq* via interaction with the transcriptional activators WC-1 and WC-2 and promotes the accumulation of a new pool of WCC through positive action on the expression of *wc-1* and *wc-2*. As levels of FRQ decrease over the course of a day, the WCC can again activate *frq* transcription and the positive and negative feedback loops of gene expression are repeated. In flies and mammals, dual actions of clock molecules promoting and repressing the activity of other clock components indicate that the emergence of circadian rhythmicity from a web of interconnected transcription/translation loops is a common theme [37]. A number of delaying steps that generate the long periodicity of clock gene expression have been uncovered. Regulation of the synthesis, stability, compartmentalization and activity of clock components can determine the rate at which each molecular cog in the clock mechanism turns. For instance in *Neurospora*, FRQ and WC-1 are progressively phosphorylated over the course of each day. If FRQ phos-

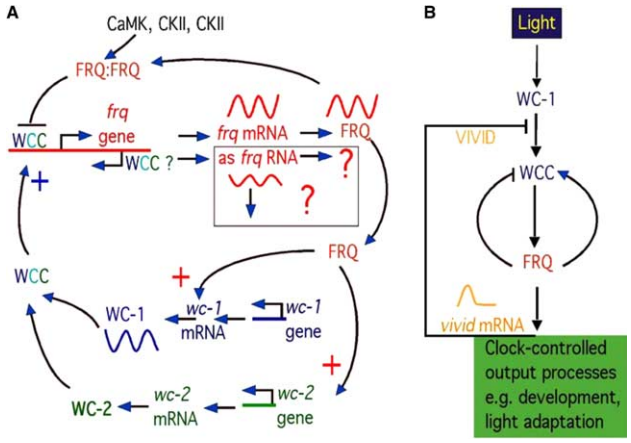

Fig. 2. Molecular components of the *Neurospora* clock. A, Simplified model of the *Neurospora* clock, showing interactions of clock molecules: the products of the *frq*, *wc-1* and *wc-2* genes. FRQ acts both to repress its own transcription and to promote the levels of *wc-2* mRNA and production of WC-1 protein. WC-1 and WC-2 form the WCC that activates transcription of the *frq* gene. Cycling transcripts and protein are shown as sine waves. Boxed area highlights unknown regulation and action of antisense (as) *frq*. Kinases known to phosphorylate FRQ are calcium calmodulin dependent kinase (CaMK) and caesein kinase I (CKI) and CKII. B, A common theme to emerge is that clock molecules are often part of or closely associated with a signalling pathway relaying light or redox information to the clock. For instance, the WC-1 protein is both a blue light photoreceptor in *Neurospora* and a transcription factor required for activation of *frq*. *vivid* encodes a photoreceptor with a role in light adaptation.

phorylation sites are mutated, stability of the protein increases and periodicity is either lengthened or abolished. Some of the kinases responsible for FRQ phosphorylation are casein kinase 1 [40] and II [41] (CKI and CKII), and a calcium/calmodulin dependent kinase (CaMK) [42]. Moreover, additional interlocking loops have been discovered from the clock or clock output pathways to gate input pathways (Fig. 2B) conferring the ability of the clock to respond to the environment only at appropriate times of the day.

Latterly the first clock molecules, or parts thereof, have been used to search for clock gene homologues in other organisms. In the mid 1990s, a motif present in four out of the five known clock components was the PAS domain. Thus proteins containing PAS domains were prime candidate clock proteins and were hunted down in sequenced genomes and the host proteins characterized for clock function. The acronym "PAS" stands for period, aryl hydrocarbon nuclear translocator (ARNT) and single-minded (SIM), the three proteins in which the domain was discovered [43]. This common motif was also used to pull out clock molecules by degenerate PCR and clock proteins themselves were used in 2-hybrid screens to pull out interactors. These approaches yielded both homologues of known clock genes and entirely new clock components [44]. For example, whilst in *Drosophila* there is one *per* gene, in mammals there are 3. The first of the mammalian *pers* was identified by degenerate PCR [45], the second and third by searching for sequence homology in silico [46,47]. In this respect, the similarity between components of fly and mammalian clocks gave a much-needed boost to mammalian clock research and resulted in an explosion in the number of known and putative clock molecules. The number of known clock and clock-associated molecules, i.e., molecules feeding time information to the clock and relaying time-of-day signals from the clock to control output processes, is now so numerous that modelling to test the possible impact of each component in the system as a whole is almost a necessity. A final proof that all the major players have been discovered will be the reconstruction of a circadian clock in vitro or in vivo in clock-less cells.

Though recent molecular data indicate that circadian clocks have arisen several times in the course of evolution [48], the best-studied clocks appear to have emerged from the interactions of pathways sensing and responding to environmental stimuli. Thus, several clock and clock-associated molecules are also integral components of light input pathways, e.g., WC-1 [28,49] and VIVID [50,51] in *Neurospora*, *CRYPTOCHROME* (*CRY*) 1 and 2 and *PHYB* in *Arabidopsis* [52] and *dCRY* in *Drosophila* [53,54]. In mammals, though *CRYPTOCHROMES* do not transduce light signals to the clock they are central clock components and essential for circadian timekeeping [37]. This together with the anatomical coincidence of light sensing organs and circadian clocks [e.g., the master clock in mammals in the supra chiasmatic nuclei (SCN) and the optic chiasm] gave credence to the idea that signalling pathways were the raw material for the evolution of circadian clocks. Other clock components, though not light regulated, nonetheless respond to the redox state of the cell [55]. Thus, an understanding of environmental sensors and their signal transduction pathways continues to shed light on clock mechanism [50,52]. To date chronobiologists have concentrated almost entirely on the role of clock gene transcripts encoding proteins. However, in at least one organism it appears that antisense clock gene transcripts play a role in the response of the clock to light [56].

3. Natural antisense RNA and possible modes of action

Antisense RNA can be defined as RNA that is complementary to another RNA. Complementary *cis*-encoded RNAs arise due to convergent or divergent transcription such that either the 3′ or 5′ ends of the transcripts are complementary. Occasionally, sense and antisense RNAs are completely overlapping [56–58]. Moreover, transcripts arising from different regions of the genome (*trans*-encoded RNAs) may share limited complementary sequence with another transcript. In a region of the genome where both strands are transcribed, the first transcript to be discovered is usually known as the sense transcript and the subsequently identified complementary transcript is then by definition antisense RNA. Though examples of regulatory non-coding and antisense transcripts have been known for quite some time, a growing pool of data suggests that such molecules are far more prevalent than was previously anticipated [59–61].

In mammals [62–64] and in plants [65] directionally cloned EST sequences have been analysed using algorithms to detect evidence of natural sense antisense pairs (SATs) and/or sequences derived from the opposite strand of an annotated gene. These efforts along with experimental protocols to identify complementary RNAs [66] have resulted in an explosion in the known and predicted antisense RNAs. It is now estimated that there are somewhere in the region of 1600 human SATs. The number of antisense RNAs has been increased still further due to the realization that the small non-coding regulatory RNAs, *lin-4* and *let-7*, discovered in *Caenorhabditis elegans*, belong to a large family of microRNAs

[67]. The active form of these small RNAs recognizes and binds to partially complementary sequences in the 3′UTRs (untranslated regions) of the heterochronic *lin-14* [68], *lin-28* [69] and *lin-41* [70] transcripts, down-regulating their translation. The similarity in size of *lin-4* and *let-7* RNAs to small interfering RNAs [71] (produced by many organisms in response to invading double-stranded RNA viruses and to some transgenes) first led to the belief that other small regulatory RNAs probably existed. Subsequently, numerous microRNAs have been identified through bioinformatic searches of DNA for the ability of putative RNA to form the characteristic hairpin loop of precursor microRNA, and by successful efforts in a number of laboratories clone the small RNAs [72]. Extrapolating from the numbers of microRNAs discovered by these approaches, it is likely that in humans at least 250 microRNAs are encoded in the genome [72].

In eukaryotes, non-coding and antisense RNA has been implicated in the regulation of a variety of important processes including RNA processing, imprinting [73], X-dosage compensation [74], antiviral responses and the regulation of translation [61]. Non-protein encoding RNA may influence gene expression through the maintenance of an open configuration in a region of chromatin allowing the easy access of DNA-binding transcription modifiers to the DNA, or by hybridization to complementary DNA or RNA [61,72]. Hybridization of complementary RNAs has been shown to block their transport from the nucleus, alter RNA sequence and its translation [61,75].

4. Clock gene antisense transcripts

To date, natural antisense RNA complementary to clock gene transcripts has been reported in mammals, insects and fungi. Due to their convergent transcription, RNAs encoding nuclear thyroid hormone receptors, *TR*α1, *TR*α2, *TR*α3 and *TR*α4 (a.k.a. ERBα1, ERBα2, etc), are partially complementary to *Rev-erb*α RNA [76] (Fig. 3A). In mammals, REV ERBα is an orphan nuclear receptor that plays an important role in the circadian clockwork regulating the expression of both BMAL1 and CLOCK [77] (two transcriptional activators that play analogous roles in the mammalian circadian system to the *Neurospora* proteins WC-1 and WC-2). The 3′UTRs of *Rev-erb*α and *Tr*α2 mRNA overlap by 269 base pairs. Whilst *Tr*α1 encodes the *TR*α thyroid receptor the other receptors, due to altered ligand binding sites, act as thyroid receptor antagonists. Though their role in vivo is not known for certain [78], evidence suggests that *TR*α2 inhibits the action of *TR*α1 by competing for thyroid hormone response elements in the promoters of thyroid regulated genes [79]. *In vitro* experiments show that as *Rev-erb*α expression increases, the ratio of *Tr*α1/*Tr*α2 increases. If this reflects the situation in vivo, specific base pairing between *Rev-erb*α and *Tr*α pre-mRNA may favour the formation of *Tr*α1 transcripts [80,81]. This led to the suggestion that regulation of alternative processing of *Tr*α1 pre-mRNA might be important in determining the responsiveness of cells to thyroid hormone [80,81]. The link to circadian rhythmicity comes not only from the fact that REV ERBα is a clock component but that transcription of *Rev-erb*α is thought to be circadianly controlled [82], at least in mouse SCN (home of the master circadian clock). If rhythmically expressed in other tissues, its expression may not only regulate transcription of mammalian clock components but in addition influence metabolic rate and development circadianly

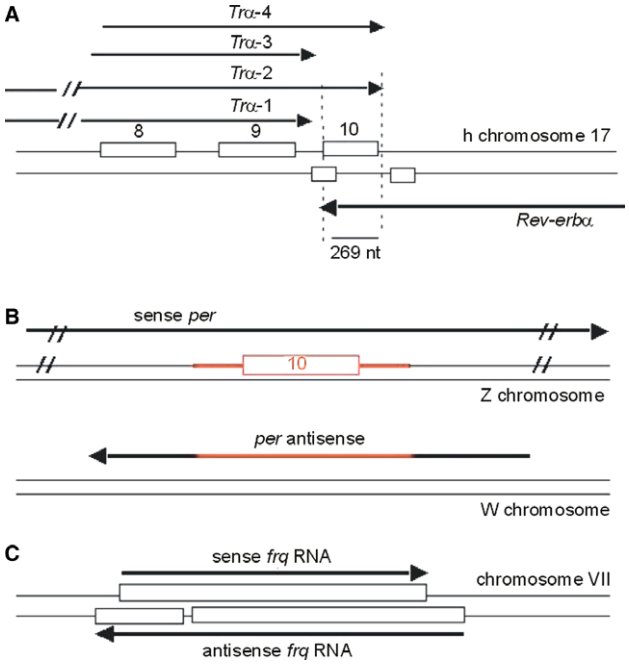

Fig. 3. Clock component antisense RNA. A, Schematic of the mouse *Trα* gene locus showing region of overlap between *Trα mRNAs* and *Rev-erbα* RNA due to convergent transcription. Several transcripts are produced from this locus due to differential splicing. Open rectangles = exons, not drawn to scale. Only *TR*α1 binds thyroid hormone, the other isoforms are antagonists, binding to DNA but having altered ligand binding sites. B, Silkmoth *per* locus on the Z chromosome encoding the clock protein PERIOD. On the female-specific W chromosome, an RNA transcribed from a *per* pseudogene produces RNA containing some complementary sequence (coloured red) to *per* mRNA. C, The *frq* locus of *N. crassa* showing the convergently transcribed and completely overlapping sense and antisense *frq* transcripts. Several sense and antisense *frq* transcripts are produced.

through the rhythmic accumulation of *Tr*α1 and *Tr*α2 RNA and protein. The latter is speculation and will depend on the location and half-life of these receptors. In adult mice *TR*α1 is reported to play a major role in regulation of heart rate and daily temperature, two physiological parameters displaying distinct circadian rhythmicity.

Another interesting example of a clock gene antisense transcript was discovered in a comparative study of *per* in the silkmoth, *Antheraea pernyi* (Fig. 3B). In *Drosophila*, the lateral neurons in the fly brain are the site of the master clock and PER enters the nuclei of these cells to repress levels of dClock and dBmal. Hence, levels of *per* transcript cycle over the course of a day. In *Antheraea*, *per* RNA is expressed cyclically as in *Drosophila* but an antisense *per* transcript is also expressed cyclically 180° out-of-phase to sense *per* RNA. Another intriguing difference is that PER protein in *Antheraea* could not be detected in the nucleus [83]. Thus, the hypothesis was put forward that regulation of PER in *Antheraea* might occur through posttranscriptional control of translation by antisense *per* RNA in the cytoplasm. However, on further investigation it was found that the antisense *per* RNA was transcribed in *cis* from a pseudogene on the female-specific W chromosome and shared only partial sequence similarity to sense *per* RNA (589 nucleotides of the 1323 nt antisense *per* transcript). Though no function of circadian or otherwise has as yet been found for antisense *per*, Gotter et al. [84] predict from its location that any role must be female-specific. Interestingly, there are now several reports of the ex-

pression of antisense RNA arising from pseudogenes or duplicated gene fragments [85,86]. For example in the snail, *Lymnaea stagnalis*, pseudogene neuronal nitric oxide synthase (nNOS) RNA is approximately 2345 nt with >80% sequence identity over at least half its length to nNOS mRNA. Though sporting a poly(A) tail, the presence of multiple stop codons indicates that this RNA is non-coding. 150 nt of the pseudogene nNOS transcript is antisense to nNOS mRNA. However, in in vitro translation assays the antisense transcript hybridizes to nNOS-encoding mRNA and prevents its translation. That the partially antisense pseudogene transcript could regulate expression of the sense RNA in vivo is supported by the observation that in neurons expressing both sense and antisense transcripts nNOS activity is suppressed [85]. The growing number of such reports bolster the suggestion that antisense *per* may at some level play a role in circadian timing.

The final example of a clock gene antisense RNA is found in *Neurospora*. In the mid 1990s, investigation of the *Neurospora frq* locus led to the identification of the 989 a.a. FRQ open reading frame (ORF) and mRNA of approximately 4.5 knt [27]. The first hint of antisense *frq* transcripts came from the sequence of *frq* cDNA clones that were non-colinear with the genomic DNA sequence, indicating the presence of an intron. On close inspection, the splice site consensus sequences were most likely to be found in an antisense *frq* transcript. Using antisense-specific riboprobes, the presence of antisense *frq* RNA was later confirmed. Several sense and antisense *frq* transcripts are convergently transcribed and completely overlapping (Fig. 3C) with the end of sense *frq* being in one case only 18 bp upstream of the antisense transcriptional start site [56]. Because of the important role of sense *frq* in the circadian clock, the levels of antisense *frq* were assayed over the course of two days in constant conditions. Like antisense *per*, levels of antisense *frq* transcripts were found to cycle in antiphase to sense *frq* RNA in the dark. Interestingly, bioinformatic searches of the sequence disclosed no large ORFs encoded in the 5–5.5 K nt antisense RNA. However, spliced antisense *frq* transcripts are also found in another filamentous fungus, *Chromocrea spinulosa*, strengthening the supposition that they are functional non-coding RNAs [56]. Though the function of antisense RNA is easily tested when the transcript encodes a protein or is transcribed in *trans*, it is difficult to tease apart the role of completely overlapping transcripts. In this situation, it is nigh on impossible to change the sequence of one transcript without altering the sequence or expression of the other. However, apart from cyclical expression of sense *frq* RNA another important property of this transcript is that its level increases rapidly in response to light and a correlation between light-induction of sense *frq* RNA and clock resetting is well established [87]. The antisense transcripts are also rapidly induced by light and this induction requires WC-1. That antisense *frq* does indeed play a role in circadian timing came from experiments in which strains lacking light-induced accumulation of antisense *frq* RNA, presumably due to deletion of WC-1 binding sites, showed a dramatic circadian phenotype.

Two strains of *Neurospora* were used to determine the importance of antisense *frq*. In the first, the *frq* 3′UTR was truncated and partially replaced with part of the 3′UTR of a transcriptionally controlled clock-controlled gene. In this strain, levels of antisense *frq* are greatly reduced in the dark and light-induction abolished. In the second strain in which ~500 bp from the 3′ end of the *frq* locus was deleted, antisense *frq* levels in constant darkness appear wild type however, again light-induction has been abolished. The 3′ ends of the sense *frq* transcripts were mapped in these strains. In the latter strain, a change in antisense *frq* has been effected without altering the 3′ end of sense *frq* transcripts. Thus, expression of the antisense RNA has been altered with no detectable effect on the expression of sense *frq* RNA. In mutant strains where light-induction of antisense *frq* RNA is eliminated, time of the internal clock is delayed relative to the wild-type strain. This is seen as a delay of molecular rhythmicity and at the phenotypic level as a delay in the clock-controlled developmental switch from vegetative growth to asexual spore formation. Moreover, in the absence of light-induced antisense transcripts it was found that the clock could be reset by 8–12 h under conditions that induce only a 1–2 h advance or delay in the wild type [56]. The data indicate a clear role for antisense *frq* in circadian timing. Because both sense and antisense *frq* transcript levels increase on exposure to light and given that the circadian clocks of strains lacking light induction of antisense *frq* show a more dramatic resetting response to light, it is possible that sense and antisense *frq* transcripts have opposing effects. As the same transcriptional activator regulates both transcripts in response to light, their antagonistic effects could be viewed as part of a mechanism that maintains the balance of active clock molecules against a background of environmental noise [56].

5. Conclusion

From the 1970s to date, clock genes have been identified in a range of organisms. Clock gene products act in interconnected positive and negative transcription/translation loops that form the molecular basis of circadian clocks. Although different molecules may form clocks in different organisms, the clocks so far studied all incorporate components of signalling pathways that sense the internal and external environment. The extensive homology of some insect and mammalian clock components and the conceptual basis of circadian clocks tested in model organisms have allowed the productive investigation into the role of circadian clocks in triggering seasonality [15] and flowering [16] and also their impact on human health [88].

Three examples of natural antisense RNA arising from clock gene loci have been described in this review. However, as the true number of natural antisense RNAs in eukaryotes becomes known it is likely that other antisense RNAs complementary to clock transcripts will be reported. In view of the evidence that antisense transcripts large and small, coding and non-coding can impact on gene expression it may prove fruitful to look again at the examples of clock gene antisense RNA mentioned above and to investigate transcripts complementary to other clock genes.

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