Title: Circadian Clocks and UPR: New Twists as the Story Unfolds.
Authors: Milev NB, Gatfield D
Journal: Developmental cell
Year: 2018 Jan 8
Issue: 44
Volume: 1
Pages: 7-9
DOI: 10.1016/j.devcel.2017.12.018

Circadian clocks and UPR: new twists as the story unfolds

Nikolay B. Milev[1] and David Gatfield[1,*]

[1] Center for Integrative Genomics, University of Lausanne, Génopode, 1015 Lausanne, Switzerland

[*] corresponding author

authors' email addresses:

nikolay.milev@unil.ch, david.gatfield@unil.ch

Summary

Circadian clocks help control the unfolded protein response (UPR). In a recent issue of Nature Cell Biology, Bu et al. (2017) show that the interaction is reciprocal, with miRNA-211 providing a signal from the UPR to the clock component BMAL1, affecting circadian timing, global translational control, and cancer cell survival.

Main text

Circadian clocks serve to anticipate the drastic daily changes in the environment and in metabolic demands experienced by most organisms. The daily gene expression oscillations found throughout organs precisely serve this purpose of temporal optimization. In some organs such as the liver, the main protein secretory activity is also gated to certain times of the day (Mauvoisin et al., 2014). High rates of protein biosynthesis can lead to stress within the endoplasmic reticulum (ER) when the fine balance between the rate of translation and the efficiency of nascent peptide folding is perturbed, and under circumstances of high oxidative or metabolic burden. When misfolded proteins accumulate, a highly conserved surveillance pathway, termed the unfolded protein response (UPR), is triggered. The UPR consists of three main branches named after their membrane-bound signal-transducers: IRE1α, PERK, and ATF6. Their activation leads to the nuclear translocation of the transcription factors XBP1, ATF4, and ATF6, which are responsible for the upregulation of genes involved in protein folding. Moreover, phosphorylation of eIF2α by PERK transiently inhibits global translation. This concerted program aims to restore organelle homeostasis or, if this fails, to trigger apoptosis.

In a recent issue of Nature Cell Biology, Bu et al. (2017) uncover a central role for the microRNA miR-211 in the complex network of interactions between the circadian system and the UPR, and demonstrate its physiological relevance in cancer. Of note, the study identifies feedback from the UPR to the clock, whereas previous studies mainly explored the reciprocal relationship. Previous studies had shown that the magnitude of drug-induced UPR in mouse liver was time of day-dependent, and that the basal, non-induced activity of signaling through the IRE1α branch of the

pathway also showed characteristic rhythms, leading to rhythmic expression of several UPR components. Additionally, the absence of a functional clock perturbed these rhythms (Cretenet et al., 2010). This and other findings have led to the notion that the circadian clock is an important player in counteracting ER stress and coordinating UPR activity with periods of high secretory demand.

But does the UPR also feed back to the clock? The starting point for the recent study by Bu et al. (2017) was the observation that drug-induced UPR delayed the phase of circadian gene expression oscillations in cultured cells and in mouse liver by several hours. Pharmacological and genetic evidence further implicated the PERK branch of the UPR in this phenotype, and molecular analyses uncovered that the phase delay coincided with a rapid and transient suppression of two core clock proteins, CLOCK and ARNTL/BMAL1. The heterodimeric transcription factor CLOCK:BMAL1 drives the activating limb in the clock's negative feedback loop. CLOCK:BMAL1 abundance is nearly constant over the day and their rhythmic activity is regulated post-translationally. Conceivably, the unusual temporary clearance of CLOCK:BMAL1 observed upon UPR activation would thus transiently halt or delay oscillations. In this paper, the authors find that the miR-211 is the key molecular player linking the UPR and CLOCK:BMAL1. miR-211 is well-positioned to play this role: unlike most liver miRNAs, which are stable (Du et al., 2014), miR-211 is labile and thus can relay transient responses (Chitnis et al., 2012). Previous work by the authors also demonstrated that this miRNA is induced through ER stress via the PERK-eIF2α-ATF4 axis (Chitnis et al., 2012). In the current study, the authors find that miR-211 targets circadian clock components in several ways. Regulation of CLOCK is accomplished in the traditional way – through post-transcriptional downregulation via a miR-211 seed site in the *Clock* 3' UTR. However, BMAL1 suppression is unusual in that it occurs at the transcriptional level, perhaps via a mechanism known as RNA induced transcriptional silencing (RITS). RITS, which involves the small RNA pathway, histone modifications, and heterochromatin formation is well-established in fission yeast and in plants (Martienssen and Moazed, 2015), and experiments presented in this current study are consistent with the regulation of *Arntl/Bmal1* by miR-211 via RITS. The functional relationship between the UPR, miR-211, and the clock is compelling: c-Myc-positive human cancer cell lines and tumors that are prone to oncogenic ER stress (e.g. Burkitt's

lymphoma), are associated with high levels of active PERK, high miR-211, and low BMAL1 levels. Importantly, inhibition of PERK or miR-211 in such cancer cell lines restores the expression of BMAL1 and of its downstream targets.

What is the function of circadian clock regulation by the UPR in tumor cells? The suppression of BMAL1 in tumors could merely represent a collateral effect of oncogenic ER stress and miR-211 induction – evidently causing a loss in clock activity, but otherwise of no further relevance for the tumor. The more exciting hypothesis would be that BMAL1 suppression is an integral part of the tumor's survival strategy. The latter indeed turns out to be the case: *In vitro*, the enforced expression of BMAL1 renders cells more sensitive to ER-stress, resulting in higher levels of apoptosis and lower survival. *In vivo*, xenograft tumor growth in mice is enhanced when BMAL1 levels are increased by overexpressing the protein, or simply by inhibiting endogenous miR-211.

Since BMAL1 fulfills functions beyond its role in the circadian clock, an important question is whether tumor cells benefit from the low BMAL1 levels as a means of removing a functional circadian system, or whether other functions of BMAL1 are important in this case. This is where the last twist to the story comes into play: First, the overexpression of a mutant variant of BMAL1 that is fully competent of driving the circadian transcriptional feedback loop, but lacks the recently described capacity of BMAL1 to interact with the translation machinery and enhance cellular translational activity (Lipton et al., 2015), does not recapitulate the tumor growth inhibitory effect. The authors' data are thus highly suggestive of a model whereby BMAL1 downregulation upon ER stress acts to turn off BMAL1's capacity to enhance translation and thus serves to keep the levels of protein biosynthesis in check, avoiding further folding stress.

Bu et al. (2017) have laid exciting groundwork for future explorations into numerous questions in the larger context of circadian clocks, translational control, and cancer. For example, whereas most previously reported interactions between miRNAs and the mammalian clockwork have more of a "fine-tuning" role, miR-211 may be a more important, *bona fide* clock regulator. Does miR-211 contribute to the daily entrainment of clocks in peripheral tissues such as the liver, in particular in response to feeding (Oyadomari et al., 2008), and possibly dependent on diet, given that high fat (Ozcan et al., 2004) and fructose (Lee et al., 2008) have been reported as potent

UPR inducers? Moreover, as the authors point out, some of the data look as if antagonizing miR-211 may be all that it takes to reactivate BMAL1 and reconstitute rhythmicity in some cancer cells. This would be spectacular, as it would mean that all other essential cogwheels of the clock are still in place despite the extensive rewiring of gene expression during cancer. Importantly, the study validates the still quite new notion that BMAL1 lives a double life and assumes cytoplasmic functions in translation (Lipton et al., 2015). How this is achieved isn't clear, especially since relative to most translation machinery components and to the bulk of cellular mRNAs, BMAL1 abundance is low, especially in the cytoplasm. How does it then act as a global translational regulator and interactor of the mRNA cap? We are clearly only at the beginning of piecing this puzzle together. And, finally, the effect of this proposed mechanism on cancer growth may expose new points of attack in the treatment of disease.

References

Bu, Y., Yoshida, A., Chitnis, N., Altman, B.J., Tameire, F., Oran, A., Gennaro, V., Armeson, K.E., McMahon, S.B., Wertheim, G.B.*, et al.* (2017). A PERK-miR-211 axis suppresses circadian regulators and protein synthesis to promote cancer cell survival. Nature cell biology.

Chitnis, N.S., Pytel, D., Bobrovnikova-Marjon, E., Pant, D., Zheng, H., Maas, N.L., Frederick, B., Kushner, J.A., Chodosh, L.A., Koumenis, C.*, et al.* (2012). miR-211 is a prosurvival microRNA that regulates chop expression in a PERK-dependent manner. Molecular cell *48*, 353-364.

Cretenet, G., Le Clech, M., and Gachon, F. (2010). Circadian clock-coordinated 12 Hr period rhythmic activation of the IRE1alpha pathway controls lipid metabolism in mouse liver. Cell metabolism *11*, 47-57.

Du, N.H., Arpat, A.B., De Matos, M., and Gatfield, D. (2014). MicroRNAs shape circadian hepatic gene expression on a transcriptome-wide scale. eLife *3*, e02510.

Lee, A.H., Scapa, E.F., Cohen, D.E., and Glimcher, L.H. (2008). Regulation of hepatic lipogenesis by the transcription factor XBP1. Science *320*, 1492-1496.

Lipton, J.O., Yuan, E.D., Boyle, L.M., Ebrahimi-Fakhari, D., Kwiatkowski, E., Nathan, A., Guttler, T., Davis, F., Asara, J.M., and Sahin, M. (2015). The Circadian Protein BMAL1 Regulates Translation in Response to S6K1-Mediated Phosphorylation. Cell *161*, 1138-1151.

Martienssen, R., and Moazed, D. (2015). RNAi and heterochromatin assembly. Cold Spring Harbor perspectives in biology *7*, a019323.

Mauvoisin, D., Wang, J., Jouffe, C., Martin, E., Atger, F., Waridel, P., Quadroni, M., Gachon, F., and Naef, F. (2014). Circadian clock-dependent and -independent rhythmic proteomes implement distinct diurnal functions in mouse liver. Proceedings of the National Academy of Sciences of the United States of America *111*, 167-172.

Oyadomari, S., Harding, H.P., Zhang, Y., Oyadomari, M., and Ron, D. (2008). Dephosphorylation of translation initiation factor 2alpha enhances glucose tolerance and attenuates hepatosteatosis in mice. Cell metabolism *7*, 520-532.

Ozcan, U., Cao, Q., Yilmaz, E., Lee, A.H., Iwakoshi, N.N., Ozdelen, E., Tuncman, G., Gorgun, C., Glimcher, L.H., and Hotamisligil, G.S. (2004). Endoplasmic reticulum stress links obesity, insulin action, and type 2 diabetes. Science *306*, 457-461.