Published in final edited form as:

Nat Cell Biol. 2017 January 31; 19(2): 79–81. doi:10.1038/ncb3460.

CPEB4 links the clock and the UPR to protect the liver

Paul C. Moore and Scott A. Oakes

Department of Pathology, Diabetes Center, and Helen Diller Family Comprehensive Cancer Center at the University of California, San Francisco, CA 94143. U.S.A

Abstract

Under misfolded protein stress, the endoplasmic reticulum (ER) activates the unfolded protein response (UPR) to restore homeostasis, or commits the cell to apoptosis. A study now uncovers how the UPR is governed by the circadian clock to adjust ER protein folding capacity to metabolic demand and protect against liver damage.

Over two decades ago, the discovery of the UPR in yeast revolutionized our understanding of how cells respond to ER stress on a molecular level¹. In the last decade, specialized roles of the UPR in complex organisms, particularly in mammals, have come into focus. Given their secretory demands, metabolic tissues not only have highly developed ER and UPR, but are also prone to ER stress-related pathologies. The β -cells of the pancreas, for example, are responsible for insulin production in response to blood glucose levels. Elevated sugar intake leads to increased secretory demand and dependence on UPR activity to maintain protein-folding homeostasis². Similarly, excessive dietary fat leads to lipid accumulation and ER stress in hepatocytes, the liver cells that regulate cholesterol levels, fatty acid oxidation, and lipoprotein synthesis^{3, 4}. In both situations, failure to moderate consumption, or dysregulation of UPR pathways in β -cells or hepatocytes, can result in the development of diabetes or hepatic steatosis, respectively. One strategy organisms have evolved to match metabolic resources to demand is circadian rhythm, and recent studies have shown that the UPR is regulated by the circadian clock^{5–7}. In this issue of *Nature Cell Biology*, Maillo *et al.* reveal that Cytoplasmic Polyadenylation Element Binding 4 (CPEB4) represents a previously unknown, rhythmically regulated branch of the UPR that protects hepatocytes⁸.

ER stress can be induced by a wide array of sources: mutations in secretory and transmembrane proteins, extensive secretory burden, elevated lipid levels, oxidative stress, and nutrient deprivation. In response to ER stress, the mammalian UPR is initiated by three ER transmembrane proteins: inositol-requiring enzyme 1 α (IRE1 α), pancreatic endoplasmic reticulum kinase (PERK), and activating transcription factor 6 (ATF6). Through their luminal domains, these proteins directly and indirectly sense increases in ER misfolded proteins. For IRE1 α and PERK, this leads to dimerization and activation of

Corresponding Author Contact Information: Scott André Oakes, M.D., Professor of Pathology, University of California-San Francisco, 513 Parnassus Avenue, HSW517, Box 0511, San Francisco, CA 94143-0511, Telephone: 415-476-1777, FAX: 415-514-3165, scott.oakes@ucsf.edu.

COMPETING FINANCIAL INTERESTS:

S.A.O. is a founder, equity holder, and consultant for OptiKira, L.L.C. (Cleveland, OH).

catalytic domains in the cytosol that propagate the stress signal through activation of transcription factors and global attenuation of protein translation. ATF6 translocates to the Golgi where it is cleaved to release its N-terminal transcription factor domain into the cytoplasm for translocation to the nucleus. The net effect of this “adaptive” UPR is upregulation of ER chaperones and degradation machinery, with concurrent reduction of total protein-folding load. If ER stress cannot be mediated, however, the “terminal” UPR signals the cell to undergo apoptosis. Although this removes unhealthy cells from the population, it can lead to disastrous consequences if too many essential cells are lost².

In mammals, the suprachiasmatic nucleus (SCN) in the hypothalamus is the seat of the central circadian clock and synchronizes peripheral clocks throughout the body. At the molecular level, this entails a negative feedback loop between the CLOCK-BMAL1 heterodimer and its transcriptional targets *Period (Per)* and *Cryptochrome (Cry)*. The 24-hour cycle driven by these factors coordinates metabolic gene expression with the feeding-fasting/day-night cycle. Consequently, loss of rhythm in organs such as the liver, pancreas, skeletal muscle, and adipose tissue upsets the energy balance by affecting production and storage of glucose and lipids⁹. Several recent publications indicate that UPR factors are both regulated by and help to coordinate circadian rhythm. For instance, IRE1 α activity in mouse liver is regulated in a rhythmic manner that protects against ER stress-induced hepatic steatosis⁵, whereas in a model of glioblastoma its RNase domain degrades the mRNA of *Per1*, a target and regulator of CLOCK-BMAL1, thereby driving tumor progression⁶. ATF4, the key transcription factor of the PERK arm of the UPR, is regulated by CLOCK-BMAL1 and mediates *Per2* oscillation in the SCN, thereby providing a regulatory switch between the central circadian clock components⁷. Considering the established role of the UPR in metabolic maintenance, this is likely just the tip of the iceberg.

CPEB4 belongs to a family of proteins that bind mRNAs containing a cytoplasmic polyadenylation element (CPE) in their 3′ untranslated region (UTR). Ultimately, binding results in 3′ poly(A) tail extension and translational upregulation of target mRNAs. *CPEB4* mRNA is rhythmically regulated in mouse liver, conferring temporal translational regulation; in the absence of CPEB4, a large number of mRNAs are transcribed, but remain untranslated until needed¹⁰. To uncover the importance of this relationship *in vivo*, Maillo *et al.* used whole-mouse and liver-specific CPEB4 knockouts. Strikingly, both old age and high-fat diet (HFD) resulted in severe hepatic steatosis and development of non-alcoholic fatty liver disease (NAFLD) in CPEB4 KO mice. Subsequent analysis showed that CPEB4 was required for translation of numerous proteins involved in ER homeostasis and CPEB4 loss resulted in mitochondrial dysfunction and defective lipid metabolism, all hallmarks of ER stress. CPEB4 KO livers were highly susceptible to ER-stress-induced apoptosis and development of NAFLD.

To examine this correlation to ER stress, Maillo *et al.* studied the kinetics of CPEB4 translation compared to known UPR markers and determined that it mirrors the upregulation of *ATF4*. Under ER stress, PERK phosphorylates eukaryotic initiation factor 2 α (eIF2 α), leading to global downregulation of CAP-dependent protein translation. However, mRNAs that contain upstream open reading frames (uORFs) in their 5′UTR, such as *ATF4*, become preferentially translated¹¹. Maillo *et al.* found that *CPEB4* mRNA contained numerous

uORFs and was translated in a PERK-dependent manner under ER-stress. Moreover, temporal analysis demonstrated that UPR-mediated activation of CPEB4 and translational upregulation of its targets was sequential. Finally, Maillo *et al.* set out to detail the rhythmic mechanism governing CPEB4 activation. Although *CPEB4* transcription was not dependent on feeding, it required a working circadian clock as shown in *Cry1/Cry2* KO mice. More importantly, the degree of CPEB4 translation, and consequently the sensitivity of the liver to ER-stress-induced apoptosis, synchronized with the rhythmic transcription of its mRNA. Integration of these signals ensures robust protection against hepatocyte ER stress at the time of greatest metabolic demand (Figure 1).

This discovery of a uniquely wired branch of the UPR changes our perception of how the body manages stress. With regard to CPEB4, there are still many unknowns. CPEB4 is downstream of eIF2 α , which is coordinated by four converging pathways, collectively called the integrated stress response (ISR), which sense ER stress, amino acid deprivation, heme depletion, and viral infection¹². Although Maillo *et al.* directly tested the involvement of the UPR through PERK, the results do not exclude the possibility that CPEB4 mediates a temporal response to a broad spectrum of stressors. Additionally, these findings raise the question of whether or not CPEB4 is a universal fixture of the UPR in mammalian cells, or if it is a tool specifically crafted to the needs of the liver or a set of tissues. Is CPEB4 ubiquitously expressed in systems that rely on circadian rhythm? Is it expendable in some tissues, but critical to survival in others? The discovery of CPEB4 function in coordinating the UPR with the circadian clock serves as a reminder that the UPR is highly intricate and still full of secrets. Regardless of ties to circadian rhythm, other uORF-containing mRNAs are prime candidates for non-canonical UPR signaling.

This breakthrough provides a tantalizing hint that the UPR and circadian rhythm are intimately linked. However, further studies are needed to determine where and how this occurs. Most of the data at hand are derived from studies on the liver, where all three branches of the UPR are required to prevent disease progression and both IRE1 α and CPEB4 appear to be under circadian control^{4, 5}. Interestingly, the work by Maillo *et al.* suggests that ATF4 mRNA levels do not follow a rhythmic pattern in the liver, whereas another report shows that they do oscillate in the SCN⁷. Such discrepancies indicate that UPR modulation may occur in a tissue-dependent manner, allowing cells to tailor stress responses to their unique metabolic needs.

Even if the UPR is not regulated by the circadian clock in all tissues, this does not undermine its importance in staving off stress-induced metabolic diseases. In addition to hepatic steatosis, which has a rapid, highly visible phenotype in UPR-deficient mouse models^{4, 5, 8}, other key metabolic tissues may require a robust UPR to maintain normal function. Previous studies have demonstrated that pancreatic β -cells are susceptible to ER stress, and that this is central to the pathogenesis of multiple forms of diabetes². Mounting research on circadian rhythm in white adipose tissue makes it an attractive target for future studies on the UPR^{9, 13}. The UPR, and particularly PERK, is emerging as a pharmaceutical candidate to combat neurodegenerative diseases and cancer^{2, 14, 15}. Moving forward, we must calibrate such UPR modulators to minimize the risks of damaging critical metabolic

systems. Only by piecing together the many roles of the UPR will we be able to effectively and safely manipulate it to our advantage.

References

1. Ma Y, Hendershot LM. The unfolding tale of the unfolded protein response. *Cell*. 2001; 107:827–830. [PubMed: 11779459]
2. Oakes SA, Papa FR. The role of endoplasmic reticulum stress in human pathology. *Annu Rev Pathol*. 2015; 10:173–194. [PubMed: 25387057]
3. Baiceanu A, Mesdom P, Lagouge M, Foulle F. Endoplasmic reticulum proteostasis in hepatic steatosis. *Nat Rev Endocrinol*. 2016; 12:710–722. [PubMed: 27516341]
4. Rutkowski DT, et al. UPR pathways combine to prevent hepatic steatosis caused by ER stress-mediated suppression of transcriptional master regulators. *Dev Cell*. 2008; 15:829–840. [PubMed: 19081072]
5. Cretenet G, Le Clech M, Gachon F. Circadian clock-coordinated 12 Hr period rhythmic activation of the IRE1alpha pathway controls lipid metabolism in mouse liver. *Cell Metab*. 2010; 11:47–57. [PubMed: 20074527]
6. Pluquet O, et al. Posttranscriptional regulation of PER1 underlies the oncogenic function of IRE1alpha. *Cancer Res*. 2013; 73:4732–4743. [PubMed: 23752693]
7. Koyanagi S, et al. cAMP-response element (CRE)-mediated transcription by activating transcription factor-4 (ATF4) is essential for circadian expression of the Period2 gene. *J Biol Chem*. 2011; 286:32416–32423. [PubMed: 21768648]
8. Maillo C, et al. *Nat Cell Biol*. This issue.
9. Yoshino J, Klein S. A novel link between circadian clocks and adipose tissue energy metabolism. *Diabetes*. 2013; 62:2175–2177. [PubMed: 23801717]
10. Kojima S, Sher-Chen EL, Green CB. Circadian control of mRNA polyadenylation dynamics regulates rhythmic protein expression. *Genes Dev*. 2012; 26:2724–2736. [PubMed: 23249735]
11. Chakrabarti A, Chen AW, Varner JD. A review of the mammalian unfolded protein response. *Biotechnol Bioeng*. 2011; 108:2777–2793. [PubMed: 21809331]
12. Taniuchi S, Miyake M, Tsugawa K, Oyadomari M, Oyadomari S. Integrated stress response of vertebrates is regulated by four eIF2alpha kinases. *Sci Rep*. 2016; 6:32886. [PubMed: 27633668]
13. van der Spek R, Kreier F, Fliers E, Kalsbeek A. Circadian rhythms in white adipose tissue. *Prog Brain Res*. 2012; 199:183–201. [PubMed: 22877666]
14. Moreno JA, et al. Oral treatment targeting the unfolded protein response prevents neurodegeneration and clinical disease in prion-infected mice. *Science translational medicine*. 2013; 5:206ra138.
15. Atkins C, et al. Characterization of a novel PERK kinase inhibitor with antitumor and antiangiogenic activity. *Cancer Res*. 2013; 73:1993–2002. [PubMed: 23333938]

Figure 1. Rhythmic transcription and UPR-mediated translation of *CPEB4* synchronize to protect hepatocytes against metabolic demand

In mouse liver, the circadian clock aligns transcription of *CPEB4* mRNA with the 24-hour day/night cycle. Concurrently, increased physical activity and feeding at night boost metabolic demand and elevate risk for hepatocyte ER stress. This leads to activation of the UPR and, in particular, preferential translation of uORF-containing mRNAs through the PERK/eIF2 α axis. Convergence of these transcriptional and translational pathways vastly upregulates *CPEB4* protein levels, resulting in a robust, well-timed ER stress response.