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Spotlight

Same Concept Different Outcomes: Sugars Determine Circadian Clock Protein Fate in Animals and Plants

Most eukaryotes have a self-sustaining circadian clock that senses environmental cues and the internal metabolic state and then imposes daily temporal organization of the physiology. The clock, by nature, regulates the daily creation and destruction of a large quantity of RNA and proteins, including those at the core of the oscillator itself. Although a transcriptional–translational feedback loop maintains the rhythmicity of the oscillator, various post-translational modifications (PTMs) of clock proteins play fundamental roles in keeping the pace of the circadian clock near 24 h. To maintain circadian clock pacing, PTMs act to ensure the rapid, efficient, and precise activation, inactivation, and finally destruction of core clock proteins (Hirano et al., 2016).

Recent work has shown that clock proteins have multiple layers of PTMs and that the interplay between different PTMs plays an important role in circadian clock function. This Spotlight article focuses on an important new study (Wang et al., 2020) showing how balances between protein glycosylation and ubiquitylation are important for adjusting the pace of the circadian clock in plants and puts these findings in the context of similar studies in animal systems. Extrapolating from what is known from animal systems, this new work in plants hints that metabolic status could be communicated to the circadian clock through glycosylation of circadian clock proteins in both autotrophic and heterotrophic organisms.

POST-TRANSLATIONAL MODIFICATION OF CIRCADIAN CLOCK PROTEINS

Phosphorylation and ubiquitylation are two PTMs that are important in all eukaryotic circadian clocks studied. Casein kinase functions in the regulation of the circadian period in both animals and plants and functions by directly phosphorylating core circadian clock transcription factors. In animals, casein kinase phosphorylates the PERIOD2 (PER2) protein, a transcription factor functioning at the core of animal circadian clocks. One outcome of PER2 protein phosphorylation is that the phospho-modified PERs are recognized by F-box proteins, β-TrCP1 and β-TrCP2, ubiquitylated, and sent to the proteasome for degradation (Narasimamurthy et al., 2018). In plants, the CASEIN KINASE 1 LIKE family of proteins regulates the circadian clock by phosphorylating core clock transcription factors PSEUDO-RESPONSE REGULATOR 5 (PRR5) and TIMING OF CAB EXPRESSION 1 (TOC1) (Uehara et al., 2019), which are recognized by the F-box protein ZEITLUPE, ubiquitylated, and subsequently degraded by the proteasome (Kiba et al., 2007). These examples nicely demonstrate that conserved PTM cascades can have the same functional outcome on circadian clock proteins.

SUGAR MODIFICATION OF CIRCADIAN CLOCK PROTEINS

Recently, work on *Drosophila* and mammalian cell culture systems has shown that protein glycosylation is also important for circadian clock function in animal systems (Kaasik et al., 2013; Li et al., 2013). *O*-GlcNAcylation in animals is mediated by a pair of enzymes, *O*-GlcNAc transferase and *O*-GlcNAcase, which have opposing functions in protein *O*-GlcNAcylation. *O*-GlcNAc transferase catalyzes transfer of *N*-acetylglucosamine to proteins, whereas *O*-GlcNAcase catalyzes the removal from proteins. Core mammalian clock proteins CIRCADIAN LOCOMOTOR OUTPUT CYCLES KAPUT (CLOCK) and BRAIN MUSCLE ARNT-LIKE 1 (BMAL1) are rhythmically *O*-GlcNAcylated by *O*-GlcNAc transferase (Li et al., 2013). This leads to increased stability of the protein through inhibition of ubiquitylation and proteasomal degradation (Figure 1). Following this study, it was shown that *O*-GlcNAc transferase competes with casein kinase to post-translationally modify the circadian clock protein PER2 at a critical site in the protein, serine 662 to serine 674 (Kaasik et al., 2013). These studies reveal that glycosylation can participate in PTM networks that conclude in altered stability of core circadian clock proteins in animals.

Recently, studies have hinted that glycosylation of circadian clock proteins may be important in plants as well (Tseng et al., 2004; Xu et al., 2017). TIME-FOR-COFFEE, a plant circadian clock regulator, is modified by *O*-GlcNAcylation, but the functional outcome and mechanism that mediate this modification are unknown. Clock protein GIGANTEA (GI) was shown to interact with a glycotransferase protein, called SPINDLY (SPY), but it is not clear if GI is a target or regulatory partner of SPY (Tseng et al., 2004). In this issue of *Molecular Plant*, Wang et al. (2020) provide molecular and genetic evidence that *O*-glycosylation plays a role in the plant circadian clock through a PTM cascade that is similar to, but also distinct from, animal systems. Previous to this work, it was revealed that there are two similar glyco-transferases in plants that have distinct sugar preference. SPY is an *O*-fucosyltransferase and SECRET AGENT (SEC) is an *O*-GlcNAc transferase (Hartweck et al., 2006; Zentella et al., 2017). Wang

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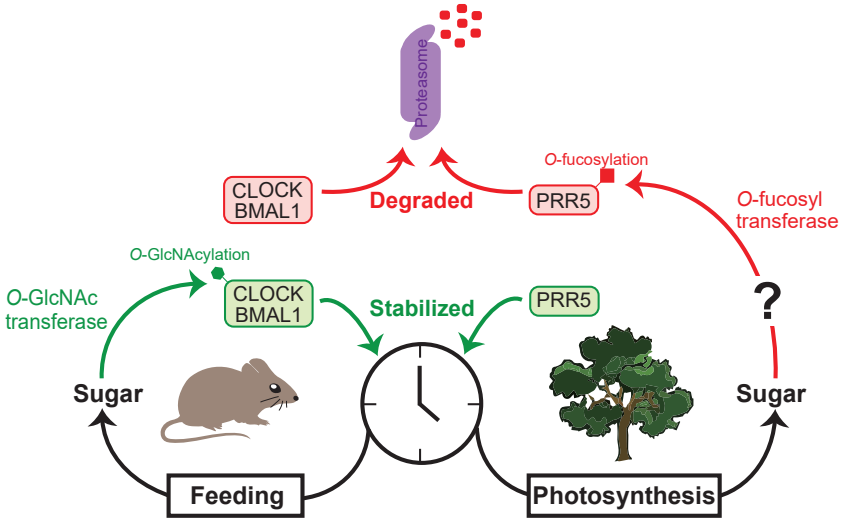

Figure 1. Roles of Circadian Clock Protein Glycosylation in Animals and Plants.
In animals, the circadian clock regulates feeding cycles, in turn controlling daily energy levels. Energy levels control *O*-GlcNAc transferase, which regulates glycosylation and increased stability of the CLOCK and BMAL1 circadian clock proteins. Plant circadian clocks regulate photosynthesis and thus energy status. Energy status can affect the circadian clock, but it remains unknown whether plant metabolic status controls the *O*-fucosylation of circadian clock proteins, akin to *O*-GlcNAcylation of circadian clock proteins in animal systems.

et al. (2020) track plant circadian rhythms in *spy* and *sec* loss-of-function mutants and demonstrate that SPY, the *O*-fucosyltransferase, but not SEC, is necessary to maintain plant circadian clock pacing. This leads them to hypothesize that plants have recruited *O*-fucosylation to control circadian clock function, distinct from animal circadian clocks, where *O*-GlcNAcylation seems to be predominant (Figure 1).

To determine the biochemical and cellular mode of action of SPY in the plant circadian clock, the authors performed a suite of protein–protein interaction studies and found that SPY interacts with PRR5, a transcription factor that is critical for circadian clock function. PRR5 is known to be modified by phosphorylation and ubiquitylation (Kiba et al., 2007; Uehara et al., 2019). Thus, the authors track the effects of SPY on the stability of the PRR5 protein. They found that SPY, and the glycotransferase activity of SPY, are necessary for the proper degradation of PRR5. This demonstrates that glycosylation and ubiquitylation can act in a PTM cascade, but the outcome in plants is opposite to that in animal systems. In animals, glycosylation can stabilize circadian clock proteins, whereas in plants, glycosylation can destabilize a circadian clock protein (Figure 1). Interestingly, it is reported that there are no *SPY-like* genes in animals (Hartweck et al., 2006), hinting that *O*-fucosylation may be mediated differently in plants. In addition, these authors are unable to show a role for SEC, the *O*-GlcNAc transferase, in plant circadian clock function. In light of previous studies that show plant circadian clock proteins can be modified by *O*-GlcNAcylation, this argues that it is not critical for basic circadian clock pacing (Tseng et al., 2004). It is clear that more work must be done to tease apart the various glycomodifications, PTM cascades, and PTM competition/cooperation events in plants and animals in future studies.

COORDINATION OF GLYCOSYLATION OF CLOCK PROTEINS IN RESPONSE TO CELLULAR ENERGY STATUS

O-GlcNAcylation is tightly regulated by the metabolic status of an organism, increasing under high-energy states and decreasing under low-energy states. In animals, this allows for metabolic entrainment of the circadian clock. Recently, it was shown that entrainment of circadian rhythms by metabolic signals is conserved in plants and animals and allows for flexibility in the timing of internal metabolic activities (Haydon et al., 2013; Kaasik et al., 2013; Li et al., 2013, Figure 1). In animals, in the presence of high glucose, PER2 *O*-GlcNAcylation increases and blocks phosphorylation and ubiquitylation, allowing for metabolic information to be communicated directly to a core circadian clock protein through a PTM cascade (Kaasik et al., 2013). In plants, metabolic status is communicated to the circadian clock through a homolog of PRR5, called PRR7 (Haydon et al., 2013). Furthermore, exogenous sucrose application shortens the circadian clock period in constant light and helps sustain oscillations in constant dark. This was shown to be dependent on the presence of GI, a protein predicted to interact with SPY, the *O*-fucosyltranferase (Tseng et al., 2004; Zentella et al., 2017). In light of the recent discovery of *O*-fucosylation of PRR5, it will be interesting to determine whether protein *O*-fucosylation links metabolic status to the plant circadian clock, akin to *O*-GlcNAcylation in animal circadian clocks. Determining the role of the metabolic status in PPR5 *O*-fucosylation would be an interesting first step.

The circadian clocks of plants and animals recruit similar cellular systems to regulate PTMs of circadian clock proteins, but distinctions remain. Circadian clocks in both autotrophic and heterotrophic organisms can be refined by PTM cascades, but the same PTMs can have different functional outcomes. The role of protein glycosylation is a clear example of this dichotomy, where circadian clock transcription factors in plants and animals are modified by sugars, but this results in opposite effects on protein stability.

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