PERSPECTIVE

O-GlcNAcylation of a circadian clock protein: dPER taking its sweet time

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In this issue of *Genes & Development*, Kim and colleagues (pp. 490–502) report that the *Drosophila* circadian repressor dPER undergoes O-linked GlcNAcylation (O-GlcNAc). Their data show that manipulation of the relevant O-GlcNAc transferase (OGT) regulates behavioral rhythmicity by affecting the stability and nuclear translocation of dPER.

Circadian clocks are biological oscillators that allow organisms to adjust behavior and physiology in anticipation of daily changes in their environment (Morrow and Brunner 2011). The *Drosophila* circadian clock is established via a transcriptional feedback loop, in which negatively acting clock proteins (dPER–TIM) rhythmically repress the activity of their positively acting transcription factors (dCLK–CYC) and thereby generate self-sustained oscillations with a period length of ~24 h. Circadian timekeeping is dependent on post-transcriptional modifications of clock proteins, including phosphorylation, sumoylation, acetylation, ADP-ribosylation, and ubiquitination, which regulate their function, localization, and turnover (Mehra et al. 2009; Asher et al. 2010).

Kim et al. (2012) show that the circadian negative regulator dPER in *Drosophila* is modified by O-GlcNAcylation, which impacts on subcellular localization and turnover of the protein and affects the circadian period length. O-GlcNAcylation also plays a role in the circadian clocks of other organisms. In *Arabidopsis*, the O-GlcNAc transferase (OGT) SPINDLY affects circadian rhythmicity (Olszewski et al. 2010). In mammals, the circadian transcription factor BMAL1 has very recently been shown to be O-GlcNAcylated, and levels of O-GlcNAc influence PER2 protein levels and the time-of-day-dependent induction of *Bmal1* gene expression (Durgan et al. 2011).

OGT targets serine and threonine residues in proteins; i.e., the same residues as most kinases. Thus, it is not surprising that in many cases the mechanism by which O-GlcNAcylation fulfills its function involves close interplay with phosphorylation (Butkinaree et al. 2010). Cross-

talk between phosphorylation and O-GlcNAcylation has been shown to occur in various ways: competitive occupancy of the same site, reciprocal or simultaneous occupancy of different sites, and site-dependent reciprocal or simultaneous occupancy (Fig. 1; Zeidan and Hart 2010). In either case, the interplay of phosphorylation and O-GlcNAcylation has the potential to generate multiple signaling states.

Circadian timing in all systems, including bacteria, fungi, plants, and animals, is intimately linked to slow, progressive hyperphosphorylation of clock proteins. The incremental increase in the phosphorylation status of clock components over the course of many hours is based on fast phosphorylation and dephosphorylation reactions that are slightly unbalanced in favor of phosphorylation (Chiu et al. 2011; Querfurth et al. 2011). Recent work by the Edery laboratory (Ko et al. 2010; Chiu et al. 2011) highlighted the importance of sequential phosphorylation of dPER for circadian timekeeping in *Drosophila*. A hierarchical cascade of phosphorylation has been shown to create a time-delay mechanism that controls dPER abundance.

The findings by Kim et al. (2012) add a new puzzle piece that might fit snugly into this mechanism of progressive phosphorylation. O-GlcNAcylation may directly block phosphorylation sites or slow down the kinetics of phosphorylation of nearby sites and thereby contribute to the characteristic kinetics of progressive phosphorylation of dPER. This notion is supported by data showing that down-regulation of the glycogen synthase kinase-3 (GSK-3), a kinase known to phosphorylate dPER (Ko et al. 2010), regulates O-GlcNAcylation of various proteins in mammalian cells (Wang et al. 2007).

In light of the finding by Kim et al. (2012), the question arises as to whether O-GlcNAcylation is intrinsically required in circadian clocks (e.g., to slow down progressive phosphorylation in order to generate an ~24-h period), or whether GlcNAcylation is a mechanism superimposed onto the clock system to allow an additional layer of regulation. Kim et al. (2012) show that down regulation as well as overexpression of OGT in *Drosophila* significantly affect period length but do not abolish rhythmicity. Hence, O-GlcNAcylation may not be essential for the clock mechanism per se, but rather may fulfill a regulatory function. As opposed to the large number of kinases and phosphatases involved in the phosphorylation and dephosphorylation of proteins, in animals, only two evolutionarily ancient enzymes, OGT and O-GlcNAcase (OGA), catalyze

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Figure 1. Possible O-GlcNAc-phosphate cross-talk on protein substrates. (A) Alternative and competitive occupancy of the same amino acid residue. Site 1 O-GlcNAcylated (G) or phosphorylated (P). (B) Alternative and reciprocal occupancy of different sites. Site 1 O-GlcNAcylated (G) or site 2 phosphorylated (P). (C) Site-dependent reciprocal or simultaneous occupancy. Site 1 O-GlcNAcylated (G) or site 2 phosphorylated (P) + site 3 O-GlcNAcylated (G). (D) No cross-talk, independent occupancy of different sites (adapted from data from Zeidan and Hart 2010).

the cycling of O-GlcNAc modification of cytosolic and nuclear proteins (Zeidan and Hart 2010). Interestingly, O-GlcNAcylation is strongly dependent on the metabolic state of a cell. A major reason for this is that the catalytic activity of OGT is regulated linearly over a wide range by the intracellular levels of its substrate, UDP-GlcNAc, which in turn reflect the nutrient status of the cell (Hanover et al. 2010). O-GlcNAcylation of specific targets modulates pathways of anabolism and catabolism, as well as cellular growth and stress responses. The series of steps leading to O-GlcNAcylation has thus been termed the hexosamine signaling pathway (Hanover et al. 2010).

Hence, O-GlcNAcylation of dPER has the potential to constitute a nutrient sensor that might enable the circadian clock to react or adapt to changes in glucose concentration. Why would this be important? Environmental cues such as temperature and nutrition impact the kinetics of biochemical reactions. Circadian clocks compensate for such effects in order to shield the molecular timekeeping mechanisms from fluctuations of such signals. Thus, all circadian clocks are temperature-compensated; i.e., their period length does not change over a wide temperature range.

Analogous to temperature, nutrients, like glucose, also impact on the period of the circadian clock. It has been shown that AMP-activated protein kinase (AMPK), a central mediator of metabolic signals, transduces nutrient signals to the circadian clock by phosphorylation of CRY1

(Lamia et al. 2009). Interestingly, transcription and expression of OGT is induced by glucose deprivation in a manner dependent on AMPK (Cheung and Hart 2008). Hence, it is tempting to speculate whether O-GlcNAcylation may contribute to metabolic compensation and/or entrainment of the circadian clock by metabolic cues. It will thus be important to localize the O-GlcNAcylation site(s) in dPER as a first step in shedding light on the functional interplay between the enzymes of O-GlcNAc cycling and the clock-relevant kinases and phosphatases.

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