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NFIL-trating the Host Circadian Rhythm—Microbes Fine-Tune the Epithelial Clock

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The diurnal activities of the intestinal microbiota and its host are closely connected, but the nature of their circadian communication pathways remains obscure. Wang et al. (2017) have described a signaling circuit linking microbial sensing by the immune system to the epithelial clock, thereby orchestrating local and systemic lipid metabolism.

The circadian organization of physiology is a critical feature of life on Earth, enabling adaptation of organismal metabolism to daily fluctuations in environmental conditions. It has recently emerged that the rhythmic activity of host physiology also comprises diurnal oscillations in the composition and function of the intestinal microbiota—the community of all microorganisms colonizing the gastrointestinal tract. These rhythms in the microbiota are driven by a functional host circadian clock and rhythmic feeding times (Liang et al., 2015; Thaiss et al., 2014; Zarrinpar et al., 2014). In turn, signals derived from the microbiome influence circadian host physiology, including transcriptional and epigenetic activity (Leone et al., 2015; Mukherji et al., 2013; Murakami et al., 2016; Thaiss et al., 2016). Thus, bidirectional communication pathways orchestrate homeostatic circadian interactions between the microbiota and the host. However, their molecular and cellular identities remain largely unknown.

In a recent publication in *Science*, Wang et al. (2017) delineate a circuit that links microbial recognition and cytokine signaling by the intestinal immune system to the epithelial circadian clock and lipid metabolism (Figure 1). The authors provide evidence that mainly gram-negative bacteria-derived signals are detected via TLRs in myeloid cells, including CD11c-expressing dendritic cells. These cells then secrete IL-23, thereby triggering a well-known pathway involving IL-22 production by type 3 innate lymphoid cells (ILCs), which in turn controls intestinal epithelial cell function (Kinnebrew et al., 2012). Using an intestinal organoid model and epithelial cells isolated by laser capture microdissection, the authors show that IL-22 stimulation of intestinal epithelial cells activates STAT3, which directly binds to the Rev-erbα promoter, reducing its expression (Wang et al., 2017). Rev-erbα is a transcriptional repressor and component of the molecular clock machinery. Microbiota-mediated reduction in Rev-erbα levels de-represses the expression of the transcription factor NFIL3, which governs a circadian transcription program of genes involved in lipid uptake and metabolism, such as *Cd36* and *Scd1* (Figure 1). Mice lacking *Nfil3* specifically in intestinal epithelial cells feature reduced levels of *Cd36* and *Scd1*, diminished amounts of serum triglycerides, and lower body fat content, reminiscent of germ-free mice. Similar to these NFIL3-deficient animals, mice with intestinal epithelial-specific loss of STAT3, or mice depleted of ILCs, exhibit reduced expression of *Cd36* and *Scd1*, further supporting the involvement of ILCs and IL-22 signaling in this pathway. Consistently, administration of either IL-22 or IL-23 to MyD88-deficient mice rescues *Nfil3* expression levels to those of control mice, bypassing the requirement of TLR- and MyD88-mediated microbiome sensing.

The generation and maintenance of rhythmic oscillations in gene expression is a complex task that requires tight coordination of epigenetic and transcriptional events along the circadian cycle and takes place in virtually all cells of the body (Koike et al., 2012). A key finding by Wang et al. (2017) is that intestinal microbial colonization is not required for rhythmic clock gene expression per se but rather tunes the amplitude of these transcriptional oscillations and thereby affects downstream gene expression, such as the metabolic program controlled by NFIL3. This does not happen in an epithelial cell-autonomous fashion but instead uses an immune cell signaling axis in the lamina propria as a relay module. The teleology of this indirect route remains unclear. The benefits of bypassing a direct microbiome-epithelial interaction in favor of a signaling circuit involving intestinal immune cells may include cytokine-mediated signal amplification and spatial distribution along the intestinal lamina propria, thereby synchronizing the amplitudes of clock gene expression along the gastrointestinal tract.

The signaling pathway involving microbial recognition via TLRs in myeloid cells, IL-23-mediated activation of type 3 ILCs, and subsequent control of epithelial cells by IL-22 is among the best-understood pathways in mucosal immunology. It mediates diverse epithelial functions in homeostasis, inflammation, and host defense, ranging from antimicrobial peptide secretion and tight junction formation to epithelial fucosylation and stem cell proliferation. The new study adds an important facet to the eclectic biology of IL-22, namely the tuning of transcriptional oscillations of the core circadian clock and its downstream genes through the regulation of Rev-erbα transcription. Importantly, the TLR-IL-23-IL-22 pathway is not shown to be intrinsically oscillating. Thus, it would be fascinating to unravel in future studies whether rhythmic levels of microbial molecules or their distribution in the gastrointestinal tract serve as an initial impulse for the generation of oscillatory activity in intestinal

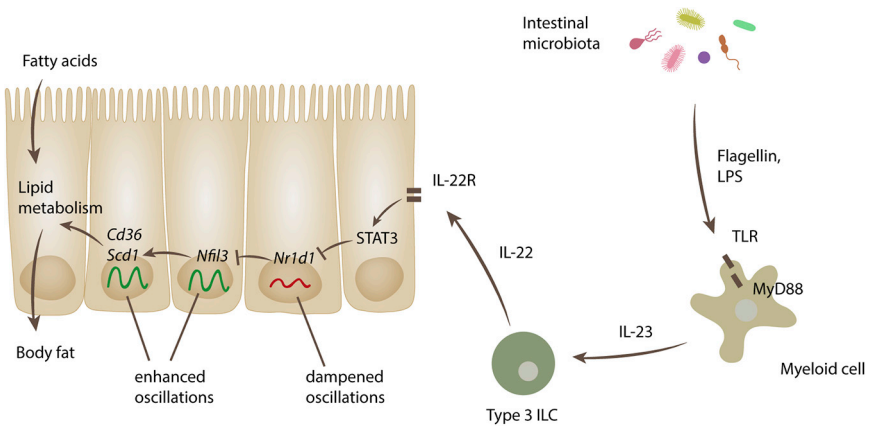

Figure 1. A Signaling Circuit Linking the Gut Microbiota to Intestinal Immune Cells and Epithelial Circadian Clock

Microbiota-derived flagellin and LPS activate TLR-MyD88 signaling on myeloid cells in the intestinal lamina propria, which triggers IL-23-mediated activation of type 3 innate lymphoid cells (ILCs). These cells, in turn, activate epithelial STAT3 signaling via IL-22. Activated STAT3 represses the transcriptional amplitude of the circadian gene *Nr1d1* (encoding Rev-erbα), thereby de-repressing the transcription of *Nfil3*. NFIL3 controls a repertoire of genes associated with lipid uptake and metabolism, including *Cd36* and *Scd1*, promoting body fat accumulation.

immune and epithelial cells, or whether they simply turn the tuning knob of constitutive immune signaling that feeds into transcriptional fluctuations generated in an epithelial-intrinsic manner. The results also raise the question of how the different consequences of IL-22 signaling are balanced, such as in the case of enteric infection, in which intestinal IL-22 levels can increase by up to 1,000-fold.

Finally, the study establishes an intriguing connection between microbiota-modulated epithelial clock activity and body fat accumulation. Disruptions of the circadian clock, as induced by shift work, jet lag, or irregular eating times, are tightly connected to the development of metabolic aberrations. Based on the findings by Wang et al. (2017), one may speculate that disturbances of diurnal microbiota activity or intestinal immune signaling lead to enhanced lipid uptake, systemic dissemination, and storage in adipose tissue. In this context, it remains to be determined whether the metabolic phenotype observed by the authors in NFIL3-deficient mice is due to the loss of oscillations in downstream metabolic genes or due to generally reduced levels of expression, i.e., whether the circadian nature of the system is critical for the synchronization of epithelial lipid metabolism to daily fluctuations in luminal nutrient levels.

These open questions notwithstanding, the study by Wang et al. (2017) provides important insights into the communication channels between microbial and host circadian activity. NFIL3-mediated lipid absorption and metabolism by epithelial cells might present an attractive target to counteract increased adiposity and dyslipidemia associated with the disruption of circadian homeostasis. While it remains unclear whether epithelial NFIL3 signaling plays a role in the metabolic complications observed in individuals suffering from jet lag- or shift work-induced circadian disruption, this study presents an example of how deciphering the diurnal crosstalk between host and microbiota on a cellular and molecular level may open new avenues for treating metabolic diseases.

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