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SPOTLIGHT

Shared circadian synchronicity in a syncytium

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In this issue, Wang et al. (https://doi.org/10.1083/jcb.202509044) report that in the syncytial fungus *Neurospora crassa*, the core proteins regulating circadian rhythms are shared between adjacent nuclei. They also show that core circadian proteins form highly dynamic nuclear bodies that oscillate in both abundance and colocalization, indicating potential for switch-like spatiotemporal regulation of circadian transcription.

Circadian rhythms are ~24-h biological oscillations that align physiological function with the rotation of the planet. These rhythms occur across eukaryotic kingdoms, from humans to the humble bread mold, and regulate diverse and critical processes, including metabolism, photosynthesis, and the sleep–wake cycle (1, 2, 3). In eukaryotes, these rhythms are regulated by a series of transcriptionally active and repressive protein complexes that translocate to the nucleus to generate rhythms in transcription. Consequently, at their most fundamental level, circadian rhythms are cellular in nature. What happens then, when you have a cell with multiple nuclei? Is time different in a syncytium?

This is the question asked by Wang et al. (4), employing the bread mold, *Neurospora crassa*. This long-established organism for circadian research employs two key factors to regulate its transcriptional circadian rhythms: the transcription factors White Collar 1 (WC-1) and White Collar 2 (WC-2), which together form the transcriptionally active White Collar Complex (WCC); and the repressor protein Frequency (FRQ). The expression of FRQ is directly driven by the WCC. FRQ then moves to the nucleus to inhibit its own transcription, forming a negative feedback loop (Fig. 1 A). While the broad strokes of this system have been defined, a few factors have made the mechanistic dissection of these proteins in *Neurospora* more difficult than their equivalents in other model circadian systems. One major factor is that WC-1 is light-sensitive, making live imaging, an invaluable technique in exploring the subcellular dynamics of proteins, impossible.

To overcome the challenge of light sensitivity, the authors designed a light-insensitive variant of WC-1 lacking the photosensitive LOV domain, thus allowing continuous imaging without light-driven resetting of the circadian clock. Additionally, they utilized mutations that slowed vegetative growth and a customized microfluidic device to slow fungal growth rate, enabling long-term and stable tracking of dynamic changes within the same nucleus. Together, these manipulations solve a long-standing issue, making longitudinal imaging of circadian protein dynamics possible in live *Neurospora crassa*. The authors verify the basic dynamics of the core circadian proteins in this system: in keeping with previous findings, the FRQ protein intensity shows periodic fluctuations over time, while the WC-2 remains almost constant (5). Thus, this ingenious system forms a reliable platform for subsequent investigation of numerous biological processes by live-cell microscopy.

Using this new biological tool, the authors next employed a heterokaryon reporter system to better understand how multiple nuclei within the shared cytoplasm of *Neurospora* synchronize circadian rhythms between nuclei. This setup employs nuclei of two different genotypes: one nucleus has all the genes required for circadian rhythms in transcription, while the other lacks FRQ but contains a luciferase reporter that responds to FRQ activity (Fig. 1 A). When isolated, the reporter nucleus is arrhythmic. However, when it is paired in a heterokaryon with a nucleus that produces FRQ, rhythmic oscillations are restored in all nuclei. Further dual-fluorescent labeling experiments showed that FRQ proteins from different nuclei mix rapidly after hyphal fusion and become evenly distributed across multiple nuclei, rather than remaining confined to the original "source nucleus." While we might typically assume that proteins mainly function in the cell nucleus close to their production site, this study showed that FRQ exhibits a highly nondirectional, nearly random distribution throughout the syncytium. This result demonstrates that core clock components are shared across the nuclei, enabling functional coupling within the syncytium. This finding opens several further questions, not least of which is whether this sharing of circadian proteins is unique to *Neurospora*, or whether it might occur in other syncytia, such as mammalian skeletal muscle cells, insect embryos, and the endosperm of plants.

The authors next employed the live-cell microscopy system to investigate the behavior of circadian proteins within single nuclei. They observed that both fluorescently tagged FRQ and WCC form nuclear foci: distinct bodies of each protein. These bodies move continuously within the nucleus with distinct dynamic behavior. FRQ foci are generally more transient and dynamic, while WCC foci are relatively more stable. The authors also showed that while

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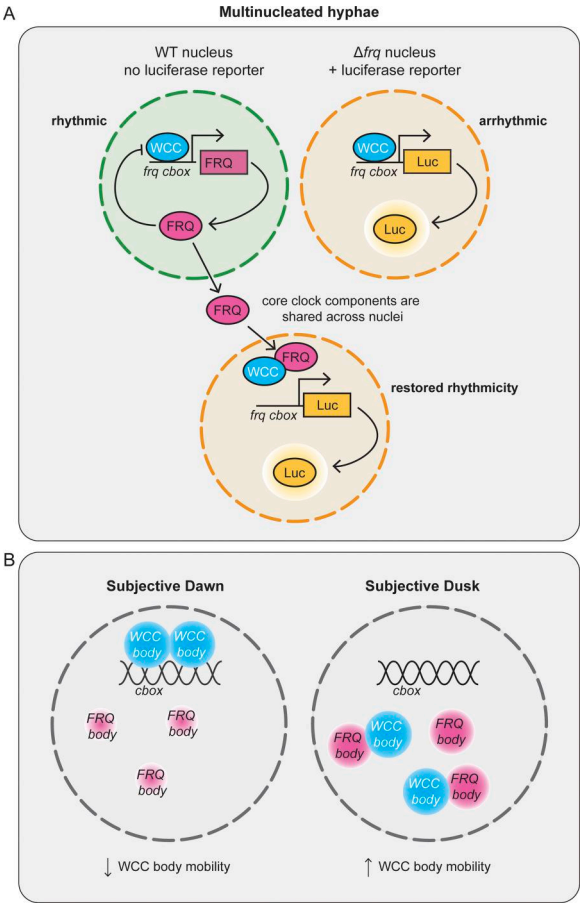

Figure 1. **Core circadian proteins are shared between local nuclei and form dynamic nuclear bodies across circadian cycles. (A)** Schematic representation of the heterokaryon reporter system. As core clock components are shared across nuclei, forming heterokaryons between nuclei with WT circadian proteins and "clockless" nuclei that lack the FRQ gene but contain a frq_{cbox}-luc reporter generates heterokaryotic hyphae with restored circadian rhythmicity in luciferase expression. **(B)** Model showing how WCC and FRQ body colocalization and mobility may correlate with subjective dawn and dusk. WCC and FRQ form dynamic nuclear bodies. At subjective dawn, WCC binds to DNA. This physical constraint reduces WCC body mobility. WCC drives the expression of FRQ. At subjective dusk, FRQ body intensity increases. WCC then binds to highly mobile FRQ bodies, releasing WCC from DNA, leading to increased WCC body mobility.

the number of FRQ and WCC foci remains constant throughout the circadian cycle, the FRQ focus intensity, but not WCC focus intensity, oscillates over time. Additionally, they observed that WCC focus behavior changes depending on whether it overlaps with FRQ. When colocalized, WCC foci were more dynamic. When not colocalized, they tended to dwell longer in a single location. Based on these observed temporal changes in FRQ and WCC nuclear dynamics, Wang et al. propose a spatiotemporal model of FRQ and WCC activity across the circadian cycle (Fig. 1 B). In this model, during the subjective early morning, WCC is less likely to bind to FRQ and is instead more likely to be situated on DNA in its role as a transcription factor. FRQ, meanwhile, exists as dispersed nuclear bodies, resulting in generally low levels of FRQ and WCC colocalization. As daytime progresses into night, FRQ accumulates in the nucleus and partially overlaps with WCC in some intranuclear structures, reaching a high point at subjective dusk. In this case, WCC is more likely to be bound by FRQ, releasing it from DNA, reducing its transcriptional activity, and making it more mobile within the nucleus. While this model requires further testing, it would align with previous work considering circadian protein dynamics in mice (6), highlighting a biophysical similarity between these diverse circadian systems.

Altogether, this work makes use of a novel system for longitudinal live-cell imaging in *Neurospora crassa* to make significant contributions to our understanding of the activities of the core circadian transcription factors in this model fungus. The new technologies and hypotheses generated by this work will stimulate new lines of inquiry that will inform our understanding of circadian biology across eukaryotic kingdoms.

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