COMMENTARY

Fungi employ the GCN2 pathway to maintain the circadian clock under amino acid starvation

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Endogenous circadian clocks enable organisms to synchronize their biological activities to the daily rhythms of light and dark, temperature and even other stressful environmental variables, which are vital for the survival of the organisms in nature. Eukaryotic circadian clocks are controlled by the transcription/translation feedback loops. In the filamentous fungus *Neurospora crassa*, this feedback loop comprises the white-collar complex (WCC) formed by two GATA-type zinc-finger transcription factors, White Collar-1 (WC-1) and WC-2, and its negative regulator FRQ protein.[1] WCC binds to the C-box of the *frq* promoter to activate its transcription and FRQ in turn interacts with other components, such as FRQ-interacting RNA helicase (FRH) and CK1, to inhibit the activity of WCC. Various stresses, including the nutritional stress, are able to disturb or even disrupt the physiological and behavioral rhythms of organisms.[2] In *N. crassa*, under amino acid starvation conditions, the serine/threonine kinase CPC-3, called GCN2 in yeast, upregulates the transcription factor CPC-1, which further regulates amino acid biosynthesis.[3] However, how the amino acid starvation stress affects circadian clock and the underpinning mechanism are still unknown.

In a recent study, Liu et al. performed a series of elegant experiments to

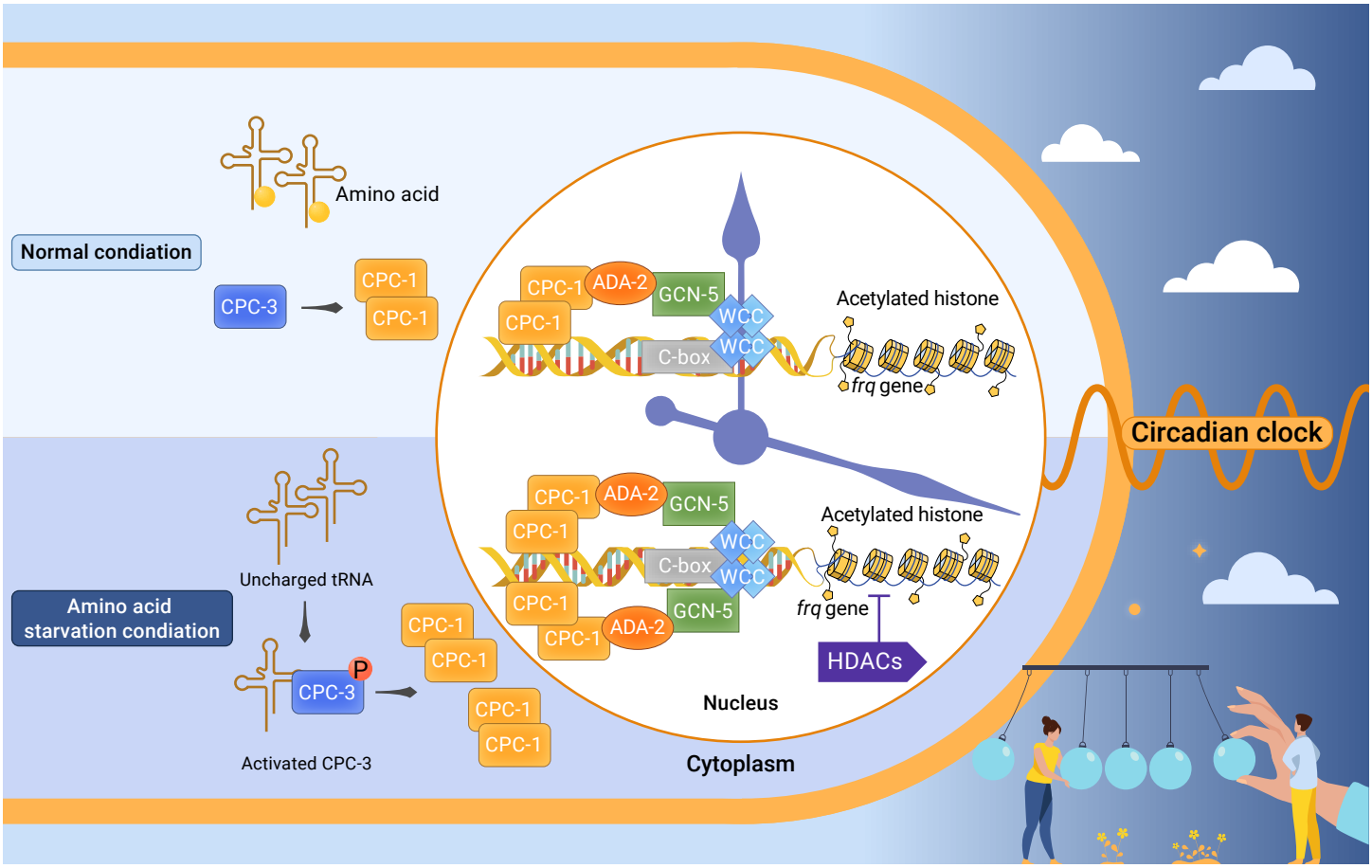

Figure 1. Schematic model for the orchestration of CPC-1, SAGA complex and WCC at the *frq* promoter under amino acid starvation stress.

unravel how the GCN2 (CPC-3) signaling pathway maintains the circadian clock functions under amino acid starvation stress.[4] They found that in the GCN2 (CPC-3) signaling pathway mutants, *cpc-3*^{KO} and *cpc-1*^{KO}, the robust circadian conidiation rhythms were inhibited in comparison to the WT strain when the inhibitor of the histidine synthesis enzyme, 3-aminotriazole (3-AT), was supplemented. The loss of circadian rhythms at molecular level was also verified by introducing the luciferase reporter under the control of the *frq* promoter into the WT and mutant strains. By performing ChIP experiments, the authors further demonstrated that CPC-3 and CPC-1 are essential for rhythmic binding of WC-2 to the *frq* promoter in response to amino acid starvation and the transcription factor CPC-1 is required for the maintenance of histone H3 acetylation (H3ac) at the *frq* promoter.

The evolutionarily conserved GCN-5 containing SAGA complex (Spt-Ada-Gcn5 acetyltransferase) is a transcriptional coactivator that regulating gene expression in part through modulating histone acetylation. By performing co-immunoprecipitation, they found that CPC-1 interacted with ADA-2 and meanwhile ADA-2 was associated with the acetyltransferase GCN-5, suggesting that CPC-1 recruits SAGA complex to modulate histone acetylation at *frq* promoter through the subunit ADA-2. In addition, the interaction between GCN5 and WCC was also proven, and the rhythmic binding of GCN-5

at the *frq* promoter was detected by ChIP experiment, which required CPC-1. These results suggest CPC-1 recruits the GCN-5 containing SAGA complex to the *frq* promoter to allow the rhythmic histone acetylation, which results in the rhythmic WCC binding and thus secures the rhythmic transcription of the *frq* gene.

To further investigate the role of GCN-5 in circadian clock functions, the *gcn-5*^{KO} strain was constructed. They found that the vegetative growth and rhythmic conidiation, FRQ abundance as well as phosphorylation after initial light/dark transition were impaired to some extent when GCN-5 was absent. Consistently, circadian rhythms of *frq* mRNA disappeared in the presence or absence of 3-AT in the *gcn-5*^{KO} strain. The results of ChIP experiments showed H3ac levels were reduced significantly and their rhythmic occupancies at the *frq* promoter were dampened in the *gcn-5*^{KO} strain. Additionally, the rhythmic binding of WC-2 at the *frq* promoter was also dramatically reduced. Taken together, these data demonstrate that GCN-5 is crucial for the circadian clock function by modulating rhythmic histone acetylation to allow the rhythmic binding of WC-2 at the *frq* promoter to control the rhythmic *frq* transcription.

Next, the authors used the histone acetylation inhibitor trichostatin (TSA) and high concentration of 3-AT to treat the WT strain and then found 3-AT lengthened the circadian period, whereas TSA slightly shortened it. In agreement with this, the longer circadian period caused by high concentration of 3-AT was restored when TSA was used together, further confirming the importance of histone acetylation for the circadian clock defects caused by amino acid starvation. The results of ChIP experiments showed H3ac levels were increased significantly when the major histone deacetylase HDA-1 that deacetylates histone H3, was absent. In contrast to the period lengthening in the WT strain caused by 3-AT, the *hda-1*^{KO} strain exhibited a normal circadian period. The arrhythmic circadian conidiation rhythm of the *cpc-3*^{KO} strain caused by 3-AT treatment was partially rescued by TSA treatment. Collectively, these results suggest that the clock defects caused by amino acid starvation in the GCN2 signaling pathway mutants are largely due to the decreased histone acetylation at the *frq* promoter.

To investigate the role of the GCN2 pathway in the regulation of gene expression under amino acid starvation, the authors performed RNA-seq experiments. 275 differentially expressed genes (DEGs) that were dependent on CPC-1 under amino starvation stress were identified. By comparing these genes with the available RNA-seq data of rhythm samples, they found that 146 DEGs were clock controlled. The results of ChIP experiments showed that CPC-1 directly bound to the promoters of amino acid synthetic genes to activate gene expression. RT-qPCR further showed that these amino acid synthetic genes were rhythmically transcribed in constant darkness under amino acid starvation conditions, which depended on CPC-1. These results demonstrate that the GCN2 signaling pathway maintains the rhythmic expression of metabolic genes under amino acid starvation conditions.

In summary, Liu et al. proved that the nutrient sensing GCN2 (CPC-3) signaling pathway maintains the circadian clock functions through modulating histone acetylation under amino acid starvation stress (Figure 1). Under normal condition, the expression of CPC-1 is kept at a basal level to recruit the GCN-5 containing SAGA complex to the *frq* promoter, which secures the acetylation of histone H3 and thus allows the rhythmic binding of WCC to the *frq* promoter. In contrast, when amnio acid is limited, histone H3 is likely kept deacetylated due to the activation of histone deacetylases (HDACs) or inhibition of histone acetyltransferases, resulting in compact chromatin structure. However, the nutrient sensing GCN2 (CPC-3) pathway is activated in response to amino acid starvation, leading to the accumulation of more CPC-1 proteins and components of the SAGA complex at the *frq* promoter, which loosens the chromatin structure to maintain the circadian clock function. Recently, in a genetic screen under glucose and amino acid starvation in *N. crassa*, a group of nutritional compensation (glucose compensation) mutants was identified, which paves the way for the future study of the nutritional compensation mechanisms.[5] If the nutrient sensing GCN2 (CPC-3) pathway is involved in nutritional compensation is worth to be elucidated in future.

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DECLARATION OF INTERESTS

The authors declare no competing interests.