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MICROBIAL PHYSIOLOGY

Optimal codons break the clock's rhythm

"both bacteria and fungi use non-optimal codon bias as a strategy to control circadian rhythm"

Circadian clocks control daily oscillations in physiological processes by coordinating gene expression with the light–dark cycle that occurs over a 24-hour period. Two studies now reveal that in the fungus *Neurospora crassa* and in the cyanobacterium *Synechococcus elongatus*, genes encoding central components of the circadian machinery display a non-optimal codon bias, and that this is an adaptation to maintain an appropriate circadian rhythm.

Codons that are decoded by abundant tRNAs (so-called optimal codons) are over-represented in highly expressed genes, which is predicted to ensure rapid and accurate translation of the mRNAs to achieve high-level protein production. The research teams began by assessing codon usage bias in circadian clock genes and found that *N. crassa frq* and *S. elongatus kaiB* and *kaiC* (which are essential for clock function in the respective organisms) exhibited non-optimal codon usage.

To evaluate the impact of codon optimization on *N. crassa* clock function, Zhou *et al.* constructed two *frq* variants: in the first, optimal codons were used in place of all the non-optimal codons in the amino terminus-encoding end of *frq* (m-*frq* construct), whereas in the second, every codon in this region was optimized (f-*frq* construct). Compared with the wild type, the FRQ levels in both the m-*frq* and f-*frq* strains were increased, but rhythmic expression of the protein was impaired. Further analysis revealed that the codon-optimized versions of FRQ were hyperphosphorylated and that they displayed heightened sensitivity to freeze–thaw cycles and trypsin digestion, all of which indicated that there were perturbations in the structure of the proteins. The authors reasoned that these perturbations were the result of improper co-translational folding of the proteins owing to faster translation of the codon-optimized *frq* mRNAs. Consistent with this hypothesis, the FRQ variant produced by the f-*frq* strain at 18 °C, rather than 25 °C, was more resistant to freeze–thaw cycles and trypsin digestion, presumably because the overall rate of translation was reduced at the lower temperature.

In the second study, Xu *et al.* found that codon optimization of *S. elongatus kaiB* and *kaiC* also resulted in increased levels of the encoded proteins; however, this did not abolish their rhythmic expression. In fact, strains carrying codon-optimized versions of these genes (optKaiB and optKaiBC strains) actually had a more robust clock than the wild type, and this was most strongly pronounced at lower temperatures (18 °C–23 °C), which this freshwater bacterium is likely to encounter in the environment.

So why have *kaiB* and *kaiC* not evolved to contain optimal codons? Xu *et al.* compared the growth rate of the wild-type bacterium with the optKaiBC strain over a range of temperatures. They found that, although the optKaiBC strain grew slightly faster than the wild type at the optimal growth temperature of 30 °C, the codon-optimized strain showed a severe growth impairment at 18 °C and 20 °C. This suggests that a robust circadian rhythm is a disadvantage at cooler temperatures. At such low temperatures, the circadian rhythm of *S. elongatus* can extend to up to 30 hours, which is predicted to disrupt the correct timing of gene expression. Thus, the bias in

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non-optimal codon usage might have evolved as a mechanism to 'condition' the circadian clock so that it is weakened in certain environmental conditions in which rhythmic gene expression might be maladaptive.

Together, these two studies reveal that both bacteria and fungi use non-optimal codon bias as a strategy to control circadian rhythm, but the outcome for each organism differs. In *N. crassa*, non-optimal codon usage seems to ensure that translation is slow enough to produce a correctly folded circadian protein, whereas in *S. elongatus*, it allows the organism to adapt the strength of circadian regulation to different environmental conditions. Finally, these data highlight the general importance of codon usage in the regulation of protein expression, structure and function.

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ORIGINAL RESEARCH PAPERS Zhou, M. *et al.* Non-optimal codon usage affects expression, structure and function of clock protein FRQ. *Nature* 17 Feb 2013 (doi:10.1038/nature11833) | Xu, Y. *et al.* Non-optimal codon usage is a mechanism to achieve circadian clock conditionality. *Nature* 17 Feb 2013 (doi:10.1038/nature11942)

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