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CELL BIOLOGY: A fable of too much too fast

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Abstract

A bacterium and a fungus both use gene sequences that fail to optimize the production of circadian-clock proteins. Two studies reveal different reasons for the advantages of producing less protein.

This is a tale of two organisms from two kingdoms that hail from very different habitats and that have nothing in common other than their ability to keep time, albeit using wholly distinct circadian systems. In this issue, Xu *et al.*[1] (page 116) and Zhou *et al.*[2] (page 111) respectively studied a freshwater cyanobacterium and a fungus. What these studies share is the fact that each organism has exploited the phenomenon of codon-usage bias to produce less of certain proteins and thereby build a more useful circadian clock. This modern fable features model organisms used in clever ways to reveal something new and confirm something old, and it reminds us that the goal of evolution is not necessarily to do more of something, but to do it better*.

First the cast of characters. The cyanobacterium is *Synechococcus elongatus*, in which three proteins, KaiA, KaiB and KaiC, self-assemble into a feedback loop that can keep time without the need for additional gene transcription or protein translation[3]. The fungus is *Neurospora crassa*, an exemplar of the circadian clocks found in fungi and animals[4] that operate using transcription-translation-based feedback loops. In these systems, heterodimeric transcription factors drive the expression of one or more 'negative-element' proteins — after a lag, these negative elements turn down the activity of their transcriptional activator. The *Neurospora* heterodimeric activator is formed from WC-1 and WC-2, and the negative element is FRQ, a protein that can be inactivated by phosphorylation at more than 100 amino-acid residues, consistent with its long-predicted propensity for inherent structural disorder[5,6].

The two research groups respectively assessed codon usage in the genes encoding KaiB–KaiC (KaiBC) and FRQ (codons are the three-nucleotide messenger RNA sequences that code for different amino acids). They found that both genes lack strong codon-usage biases, meaning that the codons used to encode the amino acids are not those that would optimize abundant protein expression. The authors demonstrate that this lack of bias is advantageous to the organism in both cases, although for different reasons.

Codon-usage bias stems from the fact that there is redundancy in the process of protein coding. Twenty standard amino acids are used to form proteins, but because the three-letter codon code gives 64 possible combinations (the four nucleotide bases taken three at a time), there is the potential for more than one codon for each amino acid. Transfer RNA (tRNA)

molecules recognize codons and carry the appropriate amino acid to the ribosome (the cellular machine that synthesizes proteins), and tRNAs can be expressed at different levels. It is thought that highly used codons correlate with abundant matching tRNA molecules, and that genes evolve to optimize the efficiency of protein translation on the rationale that faster protein synthesis yields more protein and that this is good. According to this concept, the translation rate depends on the codons used by the encoding gene, and *in vitro* studies suggest that this can not only affect the amount of protein made, but also influence the process of folding a polypeptide chain into the correct three-dimensional protein structure[7]. But until now, few studies have provided *in vivo* demonstrations of the biological significance of non-optimal codon usage.

Xu *et al*. and Zhou *et al*. sought to understand the surprising finding that neither *kaiBC* nor *frq* use optimal codons, by expressing versions of the genes in which all or some of the codons were optimized. As expected, in both cases the optimized versions made more protein, but in neither case was ‘more protein’ actually more useful.

Zhou and colleagues found that FRQ that was expressed from a gene in which either rare codons or all codons near the amino terminus of the protein were optimized could not function in the *Neurospora* clock despite the presence of adequate cellular FRQ levels. Further probing revealed that this forced codon usage induced constant, rather than rhythmic, FRQ expression, and also led to hyperphosphorylation of the protein and impaired inter actions with WC-2. This ‘optimized’ FRQ also seemed to be more sensitive to degradation by protease enzymes, leading the authors to suggest that codon optimization in *frq* leads to an increased translation rate that affects protein folding and that thereby changes FRQ structure (Fig. 1).

Interestingly, optimizing codon usage at a region that encodes the middle of FRQ and that contains many phosphorylation sites was also disruptive, yielding arrhythmic protein expression and FRQ that displayed altered protease sensitivity and inadequate phosphorylation, again indicating that proper protein folding was not attained. The reasonable conclusion from these findings is that the non-optimal codon usage for the wild-type version of this gene in *Neurospora* is a mechanism to allow the proper co-translational folding of FRQ by reducing the translation rate.

Working with *Synechococcus*, Xu *et al.* also found that optimizing codon usage in *kaiBC* led to the cells forming more protein. Surprisingly, however, codon optimization produced a more robust clock than seen in the wild type, especially when the bacteria were grown at low temperatures (which they may normally encounter). So why has the organism not evolved to optimize codon usage, if this produces a more robust clock across different temperatures? The authors show that, despite their weaker rhythm, the wild-type strain grew better under light–dark cycles (to mimic natural growing conditions) than the codon-optimized strain at low temperatures.

The explanation for this goes back to circadian first principles: clocks are evolutionarily advantageous because they help to coordinate activities in organisms such that they happen at appropriate times of day (for an example, see ref. 8). In this case, the bacterium’s natural rhythm at low temperature can be as long as 30 hours. Circadian-entrainment theory and practice both show that when a 30-h clock is entrained to a 24-h light–dark cycle, it must do so with a significantly later phase angle — so late that clock-regulated activities are driven to later and inappropriate times[9]. Thus, the temperature conditionality of the wild-type system, in which rhythms are weakened or lost at low temperatures, is good: no clock is better than a maladaptive clock (Fig. 1). This may suggest a rationale for the conditional loss

of rhythms previously reported in other organisms unable to control their internal body temperatures, including cyanobacteria, fungi[10], dinoflagellates[11] and higher plants[12].

These findings suggest that the lack of codon optimization in the two organisms probably reflects two different factors. In *Synechococcus*, *kaiBC* mRNA is extremely abundant and KaiBC is a highly structured protein, so codon optimization has its impact on the amount of functional protein that is made. By contrast, *frq* mRNA in *Neurospora* is rare and FRQ is predicted to be disordered, so the choice of codons is aimed at producing a limited amount of protein with the correct structure. But in both cases, the moral of the story is: less can be more, and quality is more important than quantity.

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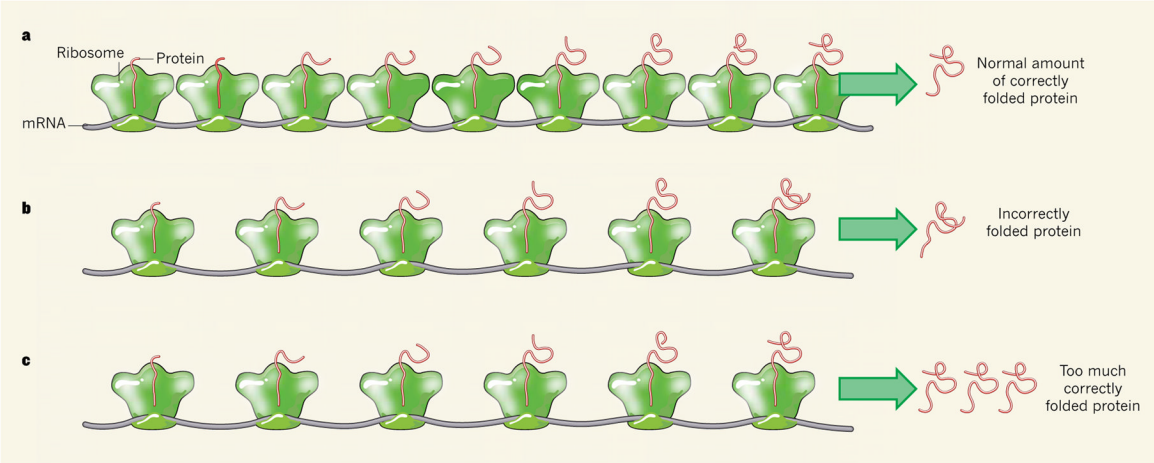

Figure 1. Balancing quality and quantity

Ribosomes are cellular machines that produce proteins by translating messenger RNA codons of three nucleotides into the corresponding amino acids. Some codons are known to facilitate more-rapid protein production than others, but it seems that producing more protein more rapidly is not always advantageous. **a**, Zhou *et al.*[2] and Xu *et al.*[1] find that both the fungus *Neurospora* and the cyanobacterium *Synechococcus* adopt non-optimal codon usage for their circadian-clock genes — *frq* and *kaiBC*, respectively. This produces normal amounts of correctly folded protein. **b**, Zhou *et al.* show that, for production of FRQ protein in *Neurospora* (assuming that the initiation of protein translation is the limiting step and that protein elongation then proceeds rapidly), forced codon optimization causes its mRNA to be translated so fast that protein folding fails, resulting in a misformed and non-functional protein that does not support a rhythm. **c**, By contrast, Xu *et al.* show that, in *Synechococcus*, optimized codon usage in the *kaiBC* gene leads to a more robust rhythm and enhanced clock-protein production (also assuming limiting initiation and rapid elongation). Surprisingly, at low temperatures, the bacterium actually grows better using the non-optimized codon usage that yields lower clock-protein production and a weakened clock. Thus, it seems that both organisms have evolved non-optimal codon usage to produce a reduced number of properly folded clock proteins that are adaptive to natural conditions.