LETTER

REPLY TO QIAN AND ZHANG:

Demonstration of the effect of codon usage on transcription by multiple approaches from fungi to animal cells

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We appreciate the interest from Qian and Zhang (1) in our recently published study on the role of codon usage in transcription in *Neurospora* (2). In our study, we demonstrate that there is a clear genome-wide positive correlation between gene codon usage biases and nuclear mRNA levels, meaning that genes with optimal codon usage have high nuclear messenger RNA (mRNA) levels. Qian and Zhang claim that we draw the conclusion of a genome-wide influence of codon usage on transcription based on this correlation (1, 2). They raise several possibilities to explain the correlation we observed, instead of the role of codon usage on mRNA levels. It should be noted that these possibilities are translation-dependent effects of codon usage. As we recently reviewed, codon usage can have important effects on gene expression independent of translation (3). The examination of nuclear RNA levels allowed us to determine the translation-independent transcriptional effect of codon usage.

Although we welcome criticism of our study, we feel strongly that the criticism raised is not warranted, because it ignores other results in the current and previous studies. In addition to the genome-wide correlation between codon usage and nuclear mRNA level, our conclusion is also based on multiple genetic, molecular, and bioinformatic results. First and foremost, by examining multiple reporter and *Neurospora* genes, we previously demonstrated that gene codon usage can have a major impact on mRNA level due to its effect on gene transcription, independent of translation and mRNA decay in *Neurospora* (4). The transcriptional effect of codon usage was indicated by altered RNA polymerase II binding, transcriptional factor binding, and chromatin markers. Second, in the current study, we show that there is a genome-wide correlation between codon usage and RNA polymerase II enrichment (2). Third, the comparison of codon optimality and its association with RNA levels between total and nuclear mRNA levels uncovers remarkable similarities (2), suggesting that a common mechanism mediates the codon usage effects on total and nuclear mRNA levels. Finally, the identification of multiple chromatin regulators in the genetic screen performed in our study shows that their deletion results in impaired codon usage effect on gene expression, altered Pol II recruitment, and reduced genome-wide correlation between codon usage and nuclear RNA levels. Together, these results suggest codon usage has a broad influence on gene transcription in *Neurospora*.

Further supporting our conclusion, we and others have also demonstrated that the impacts of codon usage bias on gene transcription and chromatin structures are conserved in *Drosophila* and human cells (5–8). A genome-wide correlation between mRNA synthesis rates and gene codon usage bias was also previously shown in yeast (9). We further show that this correlation is stronger than that between codon usage and mRNA half-life (2), suggesting that the impact of codon usage on transcription might be stronger than that on mRNA half-life (10). These results indicate the existence of a conserved eukaryotic gene regulatory mechanism mediated by gene coding sequences. Our study is the initial attempt in uncovering the molecular components in this mechanism. Future studies will determine how these factors influence transcription in a codon usage-dependent manner.

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