Sign epistasis induced by ribosome collisions during eukaryotic mRNA translation

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Ribosome stalling reduces protein expression in eukaryotes by activating quality control pathways. We used an inverse approach combining protein expression measurements with computational modeling to study the kinetics of ribosome stalling in budding yeast. Our expression measurements reveal a counterintuitive sign epistasis between mutations that cause ribosome stalling and those that decrease initiation and elongation rate. This sign epistasis is recapitulated by a kinetic model in which collision with a trailing ribosome stimulates abortive termination of a leading stalled ribosome. Our results illustrate the utility of quantitative modeling for dissecting the \textit{in vivo} kinetics of co-translational quality control in eukaryotes.

Ribosomes move at an average speed of 3–20 codons per second during translation elongation \textit{in vivo}. Since this rate is higher than the typical initiation rate of ribosomes on mRNAs [less than 1 s\textsuperscript{-1}], elongation is often assumed not to affect the expression level of most proteins. Nevertheless, the elongation rate of ribosomes can decrease significantly at specific locations on an mRNA due to low abundance of aminoacyl-tRNAs. Ribosome profiling — the deep sequencing of ribosome-protected mRNA fragments — has enabled the identification of additional factors that induce slowing or stalling of ribosomes during elongation (1). An important question emerging from these studies is the extent to which ribosome stalling affects the expression of the encoded protein, since initiation might still be the slowest step during translation.

Predicting the effect of ribosome stalling on protein levels has been challenging because of uncertainty in our knowledge of \textit{in vivo} kinetic parameters such as the duration of ribosome stalling and the rate of abortive termination at stall sites. While we have a detailed understanding of the kinetic steps and structural changes that occur during the normal elongation cycle of the ribosome, the ‘off-pathway’ events that occur at stalled ribosomes have been elucidated in only a few specific cases (2). Thus, development of complementary approaches, which can quantitatively constrain the \textit{in vivo} kinetics of stalled ribosomes without precise knowledge of rate parameters, will be useful for bridging the gap between the growing list of ribosome stall sequences and their effect on protein expression (3).

Ribosome stalling reduces protein expression in eukaryotes by activating co-translational quality control pathways (4). However the kinetic events leading to recruitment of quality control factors to stalled ribosomes have not been clearly delineated. We used an inverse approach combining protein expression measurements with computational modeling to study the kinetic events that occur upon ribosome stalling in the budding yeast, \textit{S. cerevisiae}. Surprisingly, our measurements revealed that protein expression increased from stall-containing reporters when the initiation rate or the elongation rate in the 5’ region of the mRNA was decreased through 5’ UTR or synonymous mutations. The same mutations decrease protein expression from control reporters that do not contain ribosome stalls. This counterintuitive sign epistasis is recapitulated by a kinetic model in which collision with a trailing ribosome stimulates abortive termination of a leading stalled ribosome. By contrast, canonical models of quality control without a stimulatory role for ribosome collisions do not exhibit sign epistasis. Modeling shows that sign epistasis requires multiple successive stalls and a collision-stimulated abortive termination rate that exceeds the forward elongation rate.

\textit{Hel2}, an E3 ubiquitin ligase implicated in co-translational quality control (5, 6), is necessary for the sign epistasis in our protein expression measurements, which suggests that \textit{Hel2}’s activity is specifically stimulated by ribosome collisions. Together, our results illustrate the utility of quantitative modeling for dissecting the \textit{in vivo} kinetics of co-translational quality control in eukaryotes.

\textbf{REFERENCES}