Effect of the rate of translation on the efficiency of programmed suppression

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The programmed suppression competed not only with translation termination but also with read-through suppression. During optimizing the suppression reaction conditions, we found that the rate of translation had a paradoxical effect on the exact programmed suppression reaction. The higher rate of translation does not always correlates with more efficient programmed suppression, yielding higher rate of termination at the amber codon. In fact, when we lower the reaction temperature from 37 ℃ to 25 ℃ to lower the rate of translation, the unnatural amino acid NBC was more efficiently incorporated into EPO in a cell-free protein synthesis system. Our results will present the preliminary guideline on the reaction condition for the efficient unnatural amino acid mutagenesis.