LETTER

Problems with the Analyses of the Ribosomal Allosteric Three-site Model

This letter concerns the article published recently by Petropoulos and Green (1). The detection of the third tRNA binding site, the E-site, on *Escherichia coli* ribosomes in 1981 (2) immediately raised the question of the importance of this site in addition to the established A- and P-sites. Thereafter, during almost 3 decades, the allosteric three-site model was elaborated, which defines a cross-talk between the first (A) and the third site (E) in a sense of negative cooperativity during the ribosomal elongation phase. It was based on the observation that occupation of the A-site after the decoding step releases the tRNA from E-site, and vice versa, occupation of the E-site induced a low affinity of the A-site (for review see Ref. 3). Evidence was presented that the latter effect was important for the accuracy of protein synthesis (4). We note that the allosteric three-site model is supported by a wealth of data referenced in Refs. 3 and 4.

Petropoulos and Green (1) reported that they failed to confirm the allosteric three-site model. However, they did not present one experiment addressing a possible negative cooperativity between A- and E-sites. Therefore the statement in the title is not supported by the presented evidence. Rather in the first experiment they tested our conclusion concerning initiation (unrelated to A and E allostery) that the Shine-Dalgarno interaction can functionally replace the lack of an E-tRNA (4) and demonstrated that we made a mistake in the design of one mRNA, which might explain our results. Although we agree that our conclusions using this mRNA no longer hold, we note that the second mRNA had no such error and showed a positive effect with near-cognate tRNAs, leaving it still open as to whether the original conclusion was right or wrong.

The prediction of the allosteric three-site model that an occupied E-site is beneficial for accuracy (3) could be fulfilled (4), whereas Petropoulos and Green thought to have rejected it (1). A- and particularly E-site occupancy can vary sharply depending on the ionic milieu and the ribosome preparation and thus has to be carefully controlled as we always did, whereas corresponding controls were not performed in Ref. 1. Even worse, in some experiments (Fig. 2*A* in Ref. 1) the authors applied non-fractionated, total tRNA extract, the so-called $tRNA^{bulk}$, containing one aminoacylated tRNA and a 40-molar excess of near-/non-cognate deacylated tRNAs, which we have shown previously to efficiently chase the E-tRNA (5). Therefore, the mentioned (yet not measured) ~100% E-site occupancy, which we have demonstrated in our experiments, cannot be valid here. In another experiment (Fig. 2*B* in Ref. 1) the authors mixed ribosomal subunits, deacylated tRNA, charged tRNA, and mRNA, did not use EF-G, and lacked tRNA-binding controls. Nothing can be concluded from such an experiment concerning specific effects of an occupied E-site.

In conclusion, we still believe that the allosteric three-site model has to be seriously considered and that the allosteric A-E site interactions are important for accuracy.

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