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How aminoacyl-tRNA synthetase recognizes tRNA

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Abstract

Can the selection by aminoacyl-tRNA synthetases for the correct tRNA substrate happen during binding? The results of equilibrium experiments suggest that it can.

Significance and context

In order for a cell's protein synthetic machinery to obey mRNAs to the letter, it must avoid plugging in the wrong amino acids to the nascent polypeptide chain. Part of the error-checking responsibility lies with aminoacyl-tRNA synthetases, enzymes that hook an amino acid to an adenylate group and then join it to a tRNA. The tRNAs for the different amino acids differ not only in their anticodon - which binds a complementary nucleotide triplet on the mRNA during protein synthesis - but also elsewhere in their sequences. This paper asks how aminoacyl-tRNA synthetases recognize the correct tRNA. Previous work showed that the synthetase screens tRNAs mostly during the catalysis of the amino acid-tRNA bond. Here, Bovee *et al*. ask whether an aminoacyl-tRNA synthetase also rejects 'wrong' tRNAs during the tRNA binding step. The authors test this hypothesis with equilibrium binding and tRNA footprinting experiments on *Escherichia coli* histidyl-tRNA synthetase (HisRS).

Key results

To measure binding of tRNAs to HisRS, this paper uses three different equilibrium experiments: filter binding, competition of labeled with cold substrate, and analytical ultracentrifugation. All three show that the correct tRNA (histidine tRNA) is more successful in forming stable complexes with the enzyme than a wrong tRNA (such as phenylalanine tRNA). Quantifying filter binding and competition data gives a K_d of around 4 μM for the histidine tRNA and 7 μM for the phenylalanine. When the authors add a non-hydrolyzable histidyl-adenylate analogue to the reactions, the K_d values for histidine and phenylalanine tRNAs are 1 μM and 14.5 μM, respectively. Thus, the enzyme can discriminate between tRNAs at the level of binding. The histidyl-adenylate analog, which mimics the next substrate in the enzyme's reaction, makes HisRS even more selective for tRNAs. In another section of the paper, Bovee *et al*. show results from iodine-phosphorothioate footprinting on wild-type and mutant tRNAs bound to HisRS. They find several patches of protected nucleotides on histidine tRNA, which are either spots where the tRNA binds the enzyme or intrachain RNA contacts. These hot spots change between wild-

type and mutant tRNAs. The authors are not able to use the results to pin down new insights about the HisRS mechanism, however.

Links

For a picture of the HisRS-tRNA complex see Christopher Francklyn's website.

Conclusions

Bovee *et al*. show that, at least in one case, aminoacyl-tRNA synthetases can tell the difference between the right and the wrong tRNA before they ever start catalysis. The authors also show that if the enzyme binds amino-acid-adenylate first, it is even more specific during tRNA binding. Previous kinetic data have also proven that aminoacyl-tRNA synthetases reject wrong tRNAs during catalysis, and Bovee *et al*. cite a recent model proposing that tRNA binding itself might happen in several steps - the tRNA might change conformation after its first contact with the enzyme. Specificity, therefore, seems to happen at multiple stages during the synthetase reaction.

Reporter's comments

Because amino-tRNA synthetase specificity is apparently so complicated, this paper may be probing what will prove to be a minor part of mechanism. What the field needs now is to find the rate-determining step in the synthetase reaction. That may tell us where the 'most important' specificity screen is, for both tRNA and amino-acid binding.

Table of links

Biochemistry

Christopher Francklyn's website

References

1. Bovee ML, Yan W, Sproat BS, Francklyn CS: tRNA discrimination at the binding step by a class II aminoacyl-tRNA synthetase. Biochemistry. 1999, 38: 13725-13735. 0006-2960