Quality control of translation through the kinetic discrimination of tRNAs in the network of aminoacyl-tRNA synthetases

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ABSTRACT

It is known that each aminoacyl-tRNA synthetase (aaRS) specifically recognizes its cognate tRNAs to ensure the correct translation of the genetic information. However, we had previously demonstrated that mammalian mitochondrial seryl-tRNA synthetase (mt SerRS) can markedly misacylate mitochondrial (mt) tRNA⁸⁰⁰. To investigate extensively misacylation reactions in mammalian mitochondrion, we purified overall twenty-two mt tRNAs from bovine liver, and determined their misacylation activities using mt SerRS. In addition to tRNA⁸⁰⁰, tRNA⁸⁰⁰ and tRNA⁸⁰⁰ showed weak but significant serylation activities, which raises the possibility that each mammalian mt aaRS can misacylate several non-cognate mt tRNAs in varying degrees, but translational fidelity might be maintained by kinetic discrimination of tRNAs in the network of aaRSs.

INTRODUCTION

The fidelity of protein synthesis relies on the precise tRNA aminoacylation catalyzed by aminoacyl-tRNA synthetase (aaRS). It has been shown that aaRSs strictly recognize identity elements of the substrate tRNAs, which are located within their nucleotide sequences or structural features [1].

Mammalian mitochondrial (mt) translation systems contain two serine tRNAs, each possessing an unusual secondary structure; tRNA⁸⁰⁰ corresponding to codon AGY lacks the entire D arm, whereas tRNA⁸⁰⁰ corresponding to codon UCN has an unusual cloverleaf configuration. In addition, these two tRNAs apparently share no common sequence and higher-order structure. However, we had previously demonstrated that a single mammalian mitochondrial seryl-tRNA synthetase (mt SerRS) recognizes these topologically distinct isoacceptors [2], and the ΨΨC loop region in each isoacceptor is important for the specific recognition by mt SerRS [3]. Moreover, we unexpectedly found that mt tRNA⁸⁰⁰ can be slightly but significantly misacylated by mt SerRS, probably because mt tRNA⁸⁰⁰ has a ΨΨC loop sequence identical to that of mt tRNA⁸⁰⁰ [3]. The fact that the recognition of tRNA⁸⁰⁰ by mt SerRS in vitro is 3,700 times lower than that of mt tRNA⁸⁰⁰ suggests that kinetic discrimination arising from competition between mt SerRS and mt glutaminyl-tRNA synthetase in the mitochondrion exists to maintain the fidelity of mt translation. It can be speculated that a reduction in the number of mt tRNA species might suppress the discriminatory ability of mt aaRS. Thus, our finding raised the possibility that ambiguous specificity in the recognition of tRNA is the common feature of mt aaRS, and other mt aaRSs might also misacylate non-cognate mt tRNAs.

To investigate thoroughly misacylation reactions in the mammalian mt translation system, we have purified overall twenty-two mt tRNAs, and determined their misacylation activities by mt SerRS.

RESULTS AND DISCUSSION

Native bovine mt tRNAs were purified from bovine mitochondria by selective hybridization method using a solid phase DNA probe as described by Wakita et al [4]. Because native tRNA was not fully equipped with the complete CCA sequence at
Fig. 1. Serine-accepting activities of overall 22 bovine mt tRNAs. A, aminoacylation activities of mt tRNA<sub>Ser<sup>UGA</sup></sub> (○), mt tRNA<sub>Ser<sup>GCU</sup></sub> (▲), mt tRNA<sub>Gln</sub> (◇), and other mt tRNAs were determined as described by Yokogawa et al.[2]. Each reaction was carried out in 62 µl of a reaction mixture containing 0.02 A<sub>260</sub> unit of mt tRNA substrate and 14.4 µg of mt SerRS. After defined periods of time, a 19-µl aliquot was withdrawn. B, the serine-accepting activities of mt tRNA<sub>Ala</sub> (●), mt tRNA<sub>Asn</sub> (△), and mt tRNA<sub>Met</sub> (■) (negative control). Experimental conditions were the same as those used for the plots in A.

the 3' end probably due to digestion by the endogenous nucleases, each tRNA fraction was separated by the 12% denaturing polyacrylamide gel electrophoresis after the repairing of the CCA terminus with human mt CCA-adding enzyme, and each band just obtained was cut out from the gel. Twenty-two species of native mt tRNAs were highly purified by the above-mentioned procedure.

Aminoacylation reactions using each of the native mt tRNAs and recombinant bovine mt SerRS was performed for 60 min, which was sufficient time for the degree of aminoacylation of each tRNA to reach the plateau level. Serylation activity was assayed by measuring the esterification of 14C-labeled L-serine onto mt tRNA. As shown in Fig. 1A, it was revealed that except for cognate tRNA<sub>Ser</sub>, only tRNA<sub>Gln</sub> showed marked misacylation activity, probably because among twenty non-cognate mt tRNAs, only tRNA<sub>Gln</sub> possesses the major identity determinant of mt tRNA<sub>Ser<sup>UGA</sup></sub> [3].

Moreover, in addition to tRNA<sub>Gln</sub>, tRNA<sub>Ala</sub> and tRNA<sub>Asn</sub> showed weak but significant serylation activities (Fig. 1B). The apparent serylation efficiency of tRNA<sub>Ala</sub> and tRNA<sub>Asn</sub> was ~3.3% and 2.3%, respectively. In order to ascertain the misacylation of these two tRNAs, we next performed the high sensitive assay that can directly evaluate the fraction of aminoacylated tRNA by following the nonradioactive amino acid attachment onto a 3'-[32P]-labeled tRNA [5]. By means of this method, nearly equal levels of serylation were achieved for tRNA<sub>Ala</sub> and tRNA<sub>Asn</sub>.

These results suggested that several non-cognate mt tRNAs can be misacylated in varying degrees by mammalian mt aaRS, indicating that mt translational fidelity might be maintained by the kinetic discrimination of mt tRNAs in the network of mt aaRSs. Further investigation will deepen our understanding of the quality control system in mammalian mitochondria.

REFERENCES