ABSTRACT

Transfer RNAs (tRNAs) represent an indispensable part of protein synthesis by delivering amino acids into ribosomal A site during elongation. The rules that govern the correct match between the tRNA anticodon and the A-site mRNA codon are set by the genetic code. Specifically, the code defines that 61 sense codons are recognized by tRNAs and 3 stop codons marking translation termination are recognized by the release factor. In certain circumstances, a near-cognate (or a cognate) tRNA can be incorporated at the stop codon and translation elongation proceeds further. This process, called stop codon readthrough, may be beneficial as a therapy for inherited genetic diseases caused by premature termination codons (PTCs), and importantly, it is crucial for organisms with stop codon reassignment(s).

Here I show that apart from the anticodon itself, the peculiar anticodon stem of two different near-cognate tRNAs, namely yeast *S. cerevisiae* $tRNA^{Gln}_{CUG}$ and *Blastocrithidia nonstop* $tRNA^{Trp}_{CCA}$, is critical for their readthrough promoting potential. In particular, yeast $tRNA^{Gln}_{CUG}$ relies on the specific pyrimidine 28 : purine 42 base pair forming the 4th pair of its anticodon stem, whereas $tRNA^{Trp}_{CCA}$ of *B. nonstop* acquired a mutation that shortened its anticodon stem from the canonical 5 to the 4 base pairs, i.e. the 5th pair no longer forms. Moreover, I demonstrate that *B. nonstop* evolved this shortened $tRNA^{Trp}_{CCA}$ together with the eRF1 with highly compromised UGA recognition function, due to a specific substitution of one of its conserved residues, in order to reassign UGA stop codons, highly abundant in coding sequences of its nuclear genome as UGG replacements, to the rightful amino acid Trp. Actually, in our collaborative work presented in my thesis, we also described other molecular means that *B. nonstop* uses to counteract the undesirable but absolutely fascinating appearance of all three stop codons in its coding nuclear genome.

In addition, by examining the prospective role of several small ribosomal proteins forming the decoding site in readthrough, I found that specific N-terminal residue of eS30 promote accommodation of both aforementioned tRNAs in the ribosomal A site occupied by corresponding stop codons to which they are near-cognate, most probably by contacting the top section of their anticodon stem. Furthermore, I also revealed that the N-terminal tail of eS25 specifically promotes stop codon readthrough of other near-cognate $tRNA^{Tyr}$. These results suggest that in addition to important decoding interactions stabilizing the codon:anticodon duplex, some near-cognate tRNAs can gain extra stabilization support during the naturally occurring stop codon sampling from specific interactions transiently forming between their

body and small ribosomal proteins, which are instrumental in their elevated readthrough-promoting ability.

Finally, I also participated in the development of a high throughput readthrough screening assay, which we applied to identify all efficient yeast readthrough-inducing tRNAs and to determine their interplay with various factors modulating readthrough.

Therefore, this thesis not only brings new insights into stop codon readthrough but may also contribute to the design of tRNAs with enhanced readthrough efficiency with potential application in the PTC therapy.