

Reviewer report on written Ph.D. thesis

Reviewer: Jason Chin

Candidate: Ambar Kachale

Title: Short tRNA_{CNN} anticodon stem allows C:A wobble and stop codon reassignment.

The thesis is composed of an introduction, a published paper, some perspectives and comments and conclusions, as well as copies of some other contributions. I am generally in favour of this format for a thesis as it avoids duplication of effort. I will comment on the introduction, the perspectives comments and conclusions, the paper, the candidate's contributions and then make a recommendation based solely on the written work provided.

The introduction

The introduction aims to introduce the genetic code and non-standard codes. I found the level of detail in the introduction uneven and not very well matched to the topics that constitute the experimental work. For example, there is a discussion of eukaryotic initiation, but termination does not merit a discrete section and there is minimal discussion of release factor structures, or efforts to engineer release factor specificity. Given the topic of the paper this seems like a substantive omission. Similarly, there is no discussion of synthetic code reassignment, which should be discussed alongside both natural non-standard codes, and the natural evolutionary routes that are proposed for arriving at non-standard codes.

In addition, the structure of the introduction is unclear, and the headings in the Table of contents do not seem to match the headings in the introduction. There should be a much clearer arrangement of headings and subheadings (perhaps numbered 1, 1.1, 1.2 etc.), so that the reader can follow the relationship between topics being discussed. For example, the table of contents lists *Mechanism of genetic code reassignment* on p27, but this heading does not appear on p27 and a whole series of other headings appear between p26 and 37 that are not in the table of contents. There appear to be other problems of this type in the introduction. The end of the introduction fizzles out and it is unclear what it is introducing.

Other specific comments on the introduction.

P6, Govind, is as far as I know it is commonly Gobind.

P17, The role of stop codons in code modifications may also be due to the relative tolerance to peptide C-terminal extension with respect to point mutation.

P17, Please explain the AT-mutation pressure.

P20, Near cognate decoding is discussed, but I could not find a proper discussion here or elsewhere of translational fidelity.

P21, I found the discussion of Kimura's theory in this context unclear. Please explain.

P22, Pyrrolysine is found in hundreds of organisms including archaea. Selenocysteine is in all domains of life. Please clarify. The discussion of these topics seems very light given they provide paradigms for stop codon readthrough.

P29, Please explain why you consider reassignment of UAG more complicated.

A general comment on the writing throughout: for the most part the thesis is clear, but in many places the definite or indefinite article is omitted from sentences, please carefully proofread.

The perspectives comments and conclusions

There are similar problems with the heading levels to those in the introduction, but in general this section is clear.

The paper

The paper presents interesting and important work and is generally clear and well written.

There are several nice discoveries in the paper. The key discovery is perhaps that the increase in read through of UGA stop codons is mediated by a gene that encodes tRNA^{Trp}_{CCA} with an abbreviated anticodon stem, and that this readthrough is enhanced by a release factor mutant that less efficiently terminates at UGA codons.

Some topics for discussion might include:

- 1) To what extent do the mass spectrometry methods used to look for specific tRNA modifications exclude the possibility of altered decoding mediated by modifications?
- 2) What exactly is being measured in Fig. 1e, and what is the variance of the mass spectrometry measurements?
- 3) What is the fidelity of the tRNAs with shorter anticodon stems?

The candidate's contribution

While the candidate is a first author it is unclear to me from the documents provided exactly what the candidate did. The author contribution statement in the paper is not sufficiently illuminating on this point. I suggest the author provide a description of exactly what they did and what others did (to be included at the end of the thesis) for each figure panel, extended data panels, and for any supplementary figures (the supplementary material from the paper should be included in the thesis).

Recommendation

I recommend this thesis for defense.