However, there is limited research into the presence of these specific tRNA states in the eukaryotic ribosome.

The Mindboggling Movements of Your tRNA

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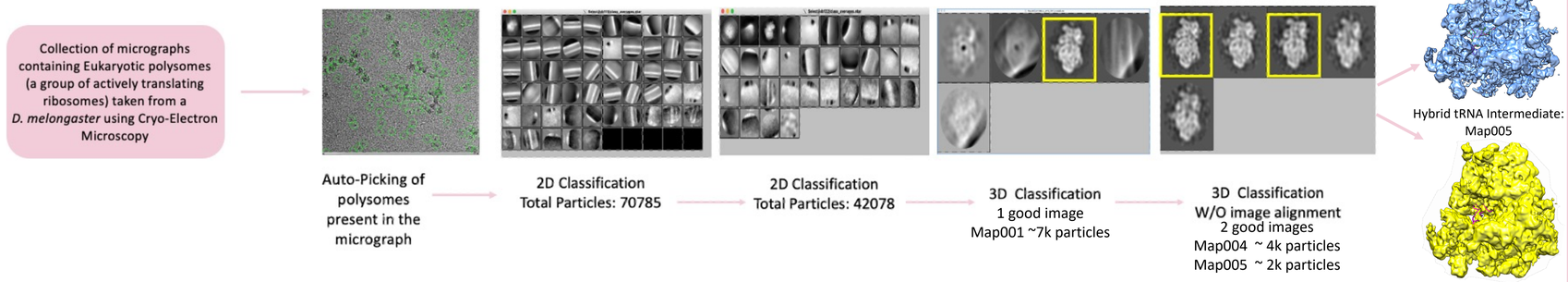
Abstract

The ribosome produces the cellular proteome converting the messenger RNA (mRNA) into a polypeptide sequence of amino acids via a transfer RNA (tRNA) intermediate. The movement of the tRNA, essential in continuing peptide synthesis, produces hybrid states of tRNA binding where the tRNA is bound to differing sites on the large and small ribosome subunit i.e., P/E and E/P. The transition between these states in the prokaryotic ribosome is incremental confirmed by Agirrezabala et al. (2012). However, there is limited research into the incremental tRNA movements in the eukaryotic ribosome. This study investigates the presence of hybrid intermediate tRNA conformations in the eukaryotic ribosomee utilizing a polysome data set taken from D. melanogaster. Two conformations, Map004 and 005, had high levels of correlating particles. Comparison of the P-tRNA density present in these conformations and the atomic structures produced by Agirrezabala et al. (2012) indicated the presence of a standard P-tRNA pdb (4v2n) and a hybrid intermediate P-tRNA pdb (4v2o). This research was limited by a small sample size reducing the number of dynamic heterogeneities captured using Cryo-Electron Microscopy but indicates the conservation of specific P-tRNA intermediate conformations between prokaryotes and eukaryotes.

Method



Introduction

- The **ribosome** is used by all living cells to produce the cellular proteome converting the **messenger RNA (mRNA)** into a sequence of amino acids via a **transfer RNA (tRNA**) intermediate.
- The tRNA moves through the ribosome in 3 stages moving from the **A site (A-tRNA)** to the **P site (P-tRNA)** to the **E site** (**E-tRNA**), before exiting the ribosome (figure 1).

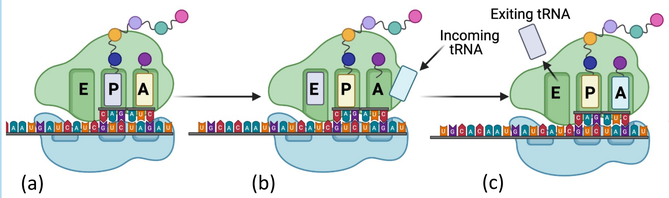


Figure 1: A diagram showing the process of an actively translating ribosome. The tRNA is accepted at the A site (a), moves to the P site where it is involved in peptide synthesis (b) before transitioning to the E site where it exits the ribosome (c). Image created using Biorender.

- The movement between the A/P and P/E sites involves **incremental transitions** across these sites producing **hybrid states of binding** (figure 2).

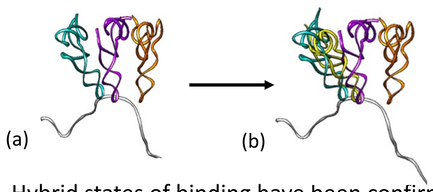


Figure 2: Image showing the standard state tRNA (a) transitioning to the hybrid intermediate tRNA state (b). The hybrid intermediate P-tRNA state pdb (4v6p) is shown in yellow. The standard P-tRNA pdb (4v6n) conformations is shown in magenta. The E-tRNA pdb (4v6w) is shown in cyan. The A-tRNA pdb (4v6n) is shown in orange. The mRNA pdb (4v6n) is shown in grey. Image created using Chimera.

- Hybrid states of binding have been confirmed in Eukaryotes (Budkevich et al., 2011) and Prokaryotes (Agirrezabala X et al., 2008); (Agirrezabala et al., 2012), however, the tRNA hybrid intermediate states have not.

Issue: Are hybrid tRNA intermediate states present in eukaryotes?

Solution: Analyse the 6 atomic maps of the Prokaryotic hybrid tRNA intermediate states, produced by Agirrezabala et al. (2012) in reference to the eukaryotic ribosome.

Results

- Comparisons of the density in Maps 004 and 005 indicated differences at the elbow region of the tRNA (figure 3).

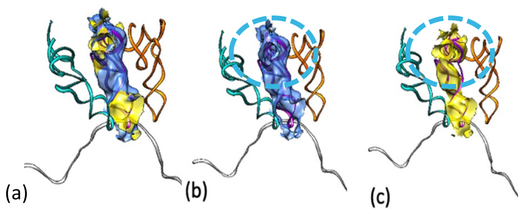


Figure 3: Image showing the Differing P-tRNA densities between Map004 (b) (Blue) and Map005 (Yellow) in relation to the standard site P-tRNA pdb (4v6n) in pink, E-tRNA pdb (4v6w) in cyan, A-tRNA pdb (4v2n) in orange and the mRNA pdb (4v6n) in grey. Figure 3(a) shows the two densities together. Figure 3(b) and (c) individually show the density in Map004 and 005 respectively. Created using Chimera.

- The density in Map004 correlated with the standard P-tRNA site map, pdb 4v2n (figure 4).

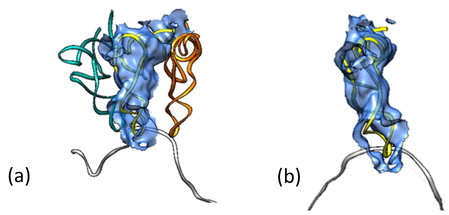


Figure 4: Image showing the P-tRNA density of Map004 (Blue) in relation to the standard P-tRNA site pdb (4v2n), E-tRNA pdb (4v6w) in cyan, A-tRNA pdb (4v2n) in orange and the mRNA pdb (4v2n) in grey. Figure 4(a) shows the density in relation to the all the tRNA sites. Figure 4(b) shows the density in relation to the standard P-tRNA and mRNA only. Created using Chimera.

- The density in of Map005 correlated with the P-tRNA hybrid intermediate map pdb 4v2o, specifically the left-rotated elbow region (figure 5).

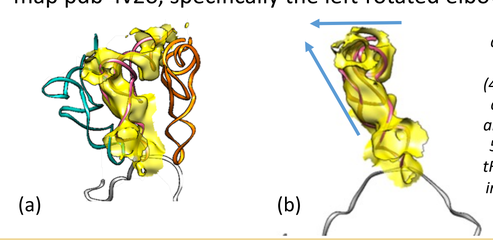


Figure 5: Image showing the P-tRNA density of Map005 (Yellow), relative to the Hybrid Intermediate P-tRNA pdb (4v6o) in Pink, the E-tRNA pdb (4v6w) in cyan, the A-tRNA pdb (4v6o) in orange and the mRNA pdb (4v6o) in grey. Figure 5(a) shows the density in relation to all tRNA sites. Figure 5(b) shows the density in relation to the Hybrid Intermediate P-tRNA and mRNA only. Created Using Chimera.

Discussion

Conclusion

Hybrid tRNA intermediate conformations are present in the eukaryotic ribosome.

Limitations

- Small sample size limited the number of tRNA intermediate conformations captured

Future Improvements

- Increase ribosome heterogeneity captured
  - Increase sample size during cryo – electron microscopy work
  - Increase number of classes used

Acknowledgements

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Key References:

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