Closing the Factory: Structurally investigating how plant CPuORFs induce ribosome stalling

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Abstract

- Conserved peptide upstream open reading frames (CPuORFs) act as a mechanism of translational regulation
- Cryo-electron microscopy (Cryo-EM) identified that fungal CPuORFs act on the ribosomal exit tunnel (RET) to induce ribosome stalling
- There is a lack of Cryo-EM information on plant CPuORF stalled ribosomes
- High resolution ribosome models were produced but CPuORFs interaction was not observed

1. Introduction

- Ribosomes are critical in **translational regulation**
- Upstream ORFs passively induce **ribosome stalling**, preventing re-initiation and translation of the main ORF
- CPuORFs **are evolutionary ancient** and conditionally regulate mORFs, including via metabolite dependent mechanisms
- The **RET** has roles in a range translational regulation mechanisms
- **Cryo-EM** was used to identify how **fungal CPuORFs interact with the RET** to induce ribosome stalling by a metabolite dependent mechanism
- **Mutagenesis** experiments suggests that plant CPuORFs interact with the RET via a metabolite dependent mechanism (Fig 1)

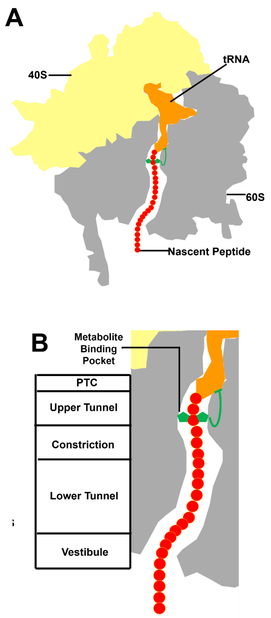
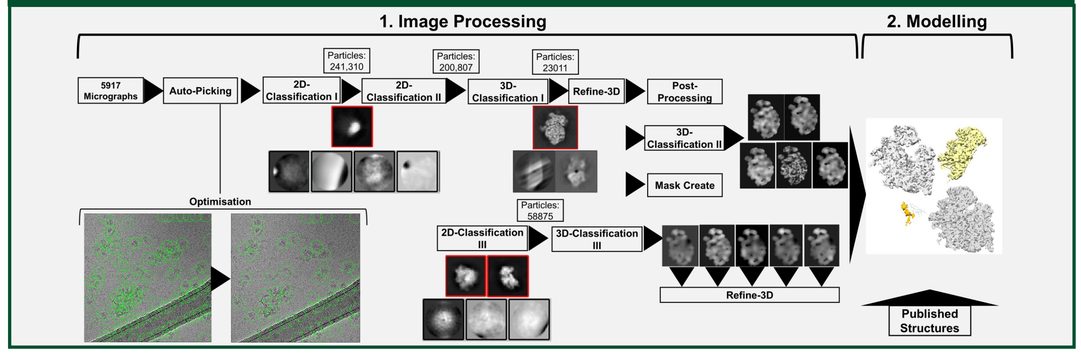


Fig 1:Hypothesised model of plant CPuORF ribosome stalling

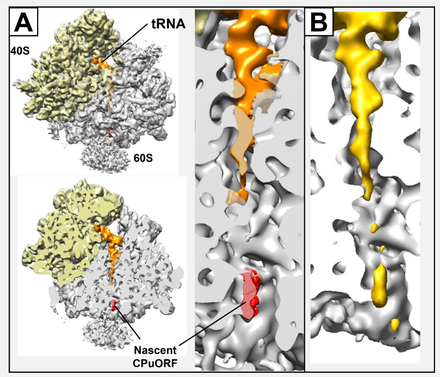
- **Aim:** Investigate CPuORF interactions in stalled plant ribosomes through producing 3D models of this complex using Cryo-EM

2. Methods

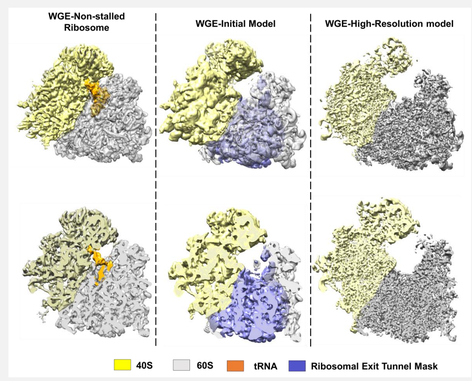


3. Results

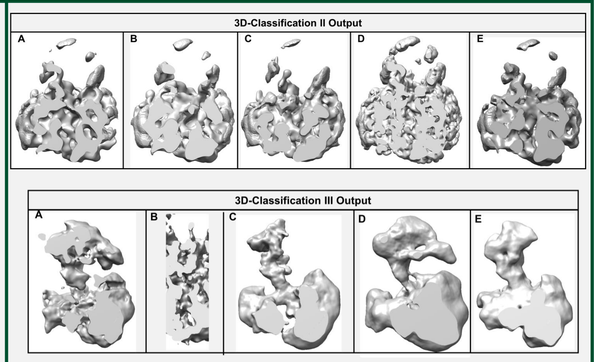
3.1 Characteristics of fungal CPuORF stalled ribosome confirmed



3.2 WGE Ribosome models do not include a tRNA-PTC interaction



3.3 All secondary 3D classifications could not resolve nascent CPuORF



- Identified **PTC-tRNA interaction** and nascent **CPuORF interaction with the lower RET** in a published fungal structure (Fig 3.1)
- Actively translating WGE negative control ribosome includes **a PTC-tRNA interaction** whereas the 4.4Å model produced here **does not contain a tRNA, nor nascent CPuORF peptide** in the RET (Fig 3.2)
- **3D classification II and III display no nascent CPuORF** in the RET however, densities are present in the lower region of 3D classification III.

4. Discussion

- Generation of 3D models of stalled WGE ribosomes **failed to resolve any nascent CPuORF peptide** in the RET. This suggests that if nascent CPuORF is present it is only present in a small fraction of ribosomes
- Future work should aim to produce a higher number of CPuORF containing ribosomes via **modification of sample preparation** methodology and **more lenient 2D and 3D-classification**
- Secondary 3D-classification III (Fig 3.3) and reduction of thresholding in the high resolution WGE model **suggests may be tRNA is present**. **Optimisation of classification steps** will allow ribosome averages to confidently confirm this
- A **density is present at the vestibule** of 3D-classification III B,D and E (Fig 3.3) however the **RTC could not be clearly defined** with resolutions ranging between 10-22Å, improving this to the ~6Å commonly achieved in published structures may assist in resolving the RTC

5. Conclusions

No nascent CPuORF peptide in the RET was identified in the models generated here, differing from published CPuORF stalled ribosome models.

Future work should aim to increase capture of CPuORF in ribosomes via optimisation of both sample preparation and in silico methods

Acknowledgements

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References

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Poster No