Investigating Apicoplast RNA polymerase gene duplication in *P. falciparum* through immunofluorescence microscopy

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This projects aims to provide initial evidence of duplicated α subunits expression and localisation, which may be essential for apicoplast activities

Introduction

Apicoplast is a nonphotosynthetic plastid which is essential for *Plasmodium falciparum's* survival that causes malaria(1)(3).

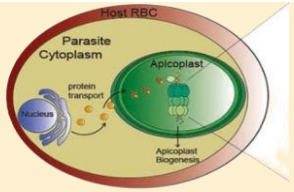

Figure1: The position of the apicoplast in *P. falciparum(3)*

Two nuclear-encoded α subunits of RNA polymerase, RpoA1 and RpoA2, from *P. falciparum* are expected to contribute to the regulation of apicoplast transcription(1).

Methods

SDS-PAGES

Cell lysis and immunoprecipitation(IP) with anti-alpha14 antibodies were performed for SDS-PAGES analysis. Following electrophoresis, sliver staining was carried out to increase sensitivity of protein detection. Then, mass spectrometry (MS) was used to verify the presence of proteins.

Immunofluorescence Microscopy

E. coli was cultured at 37 °C used as a protein factory. Affinity purification was then used to collects the duplicated alpha subunits, and the purified recombinant proteins were used to raise antibodies for immunofluorescence microscopy later. Alexa Fluor 594 and GFP fluorescence were used to detect the localisation of the alpha subunits and apicoplast respectively.

Results

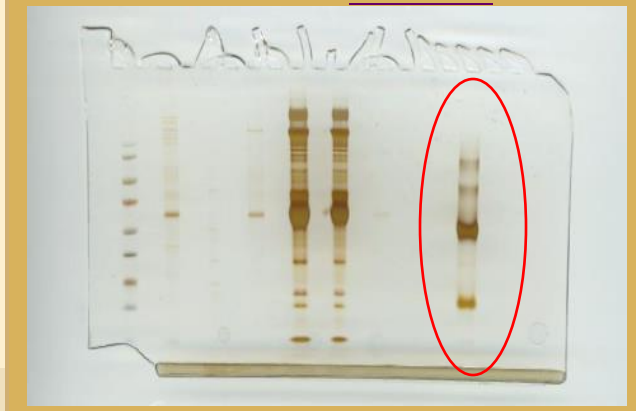

Figure2: Sliver staining of *P. falciparum, showings 2 potential bands of alpha subunits, 100 and 130kDa*

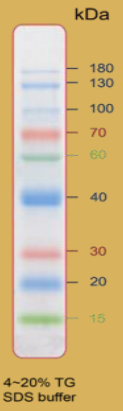

Figure3: prestained protein ladder for 15-180kDa(2)

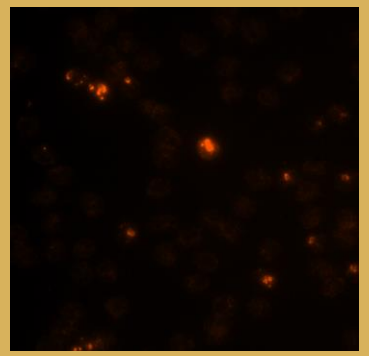

Figure4 : Immunofluorescence microscopy of RNA polymerase α-13 subunits.

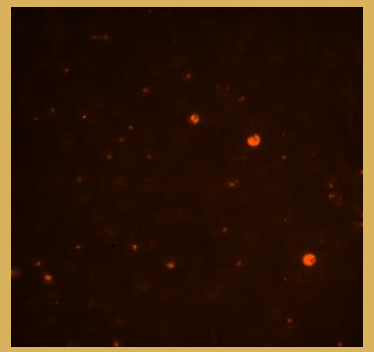

Figure5: Immunofluorescence microscopy of Negative Control(NC)

Discussion

In Figure2, heavy(50kDa) and light(25kDa) antibody chain bands and additional bands at approximately 100kDa and 130kDa were shown on the gel block. However, very low-confidence peptide matches were identified in the MS analysis of these gel bands and in-solution samples, indicating that these bands were unlikely to be the targeted proteins. Possible reasons were the low abundance of the samples or comparison with incorrect reference database.

Similar red fluorescence patterns were observed in both α-13 antibody-stained (Figure4) and negative-control slides (Figure5). This suggests that the detected red signals were likely due to autofluorescence from *P.falciparum* rather than antibody-specific binding. This may have resulted from limitations in antibody specificity or low protein expression levels. Hence, no convincing evidence of duplicated α-subunit localisation within the apicoplast was found.

Conclusion

The current results do not support localisation of the duplicated RNA polymerase α subunits of *P. falciparum* within the apicoplast. Further studies or techniques are needed such as co-staining to confirm subunit localisation.

References

1. Kobayashi Y, Komatsuya K, Imamura S, Nozaki T, Watanabe Y, Sato S, et al. Coordination of apicoplast transcription in a malaria parasite by internal and host cues. Proceedings of the National Academy of Sciences. 2023 Jul 5;120(28).
2. Prestained Protein Ladder [Internet]. US Biological Life Science; 2023. Available from: https://www.usbio.net/promos/protein-ladder
3. Tarique M. Drug Targets for Plasmodium Falciparum: Historic to Future Perspectives. Springer Nature,2024