Identifying the key players of the *Plasmodium falciparum* exportome.

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Abstract

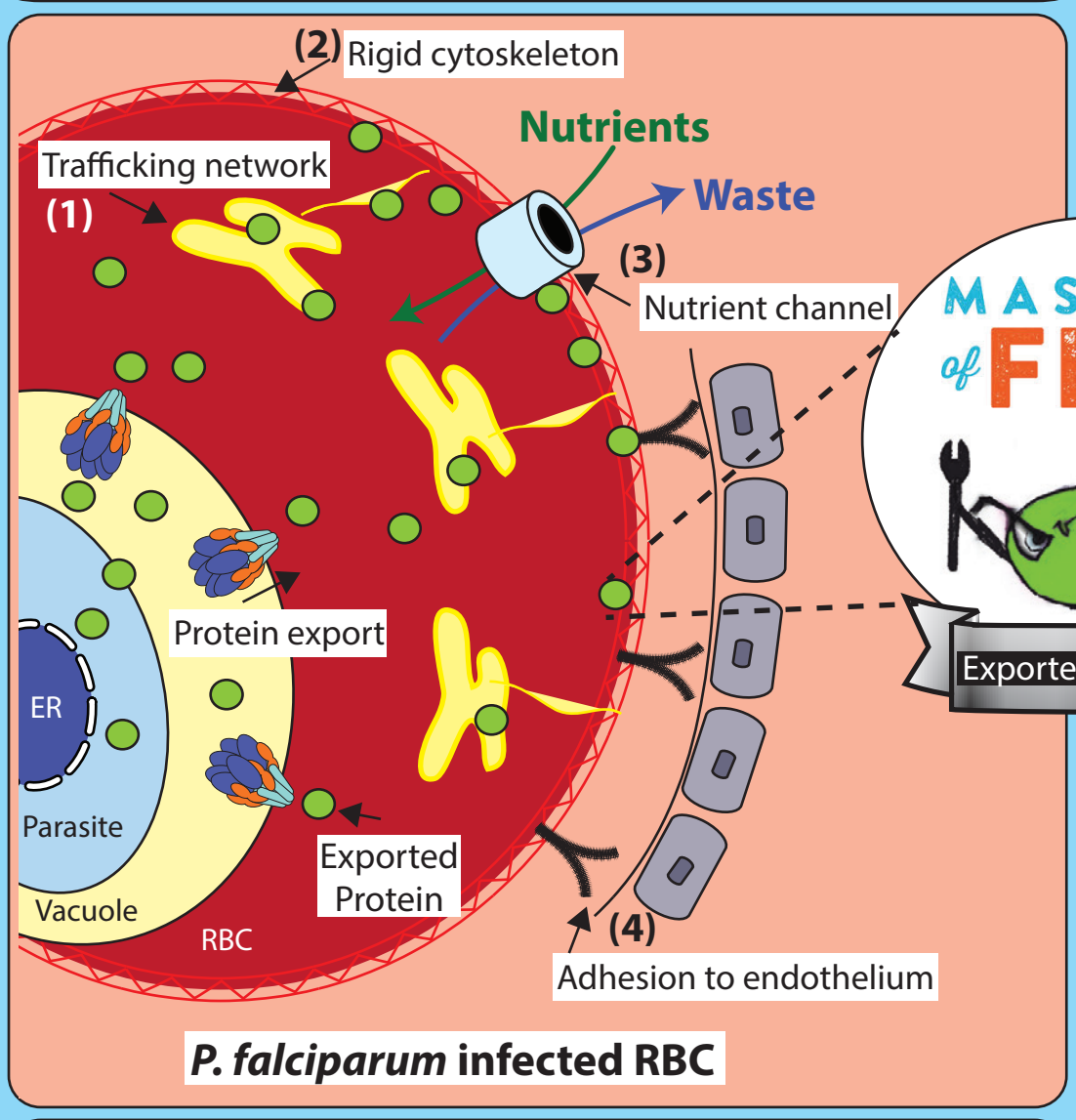
Plasmodium falciparum exporsts ~500 proteins during its blood stage. It has been estimated that 20% of these exported proteins are essential for parasite survival, however, the functions of the majority of these proteins have not been experimentally determined. Through bioinformatics analysis I have identified 63 exported proteins likely to be essential, whereby 44 have not been previously studied. I aim to study 10 of 44 proteins (6 of which are pan *Plasmodium*) using an inducible knockdown system and clarify their roles. These proteins could represent an untouched reservoir of the 'druggable proteome' and clarifying their essential roles could help form future drug targets.

Introduction

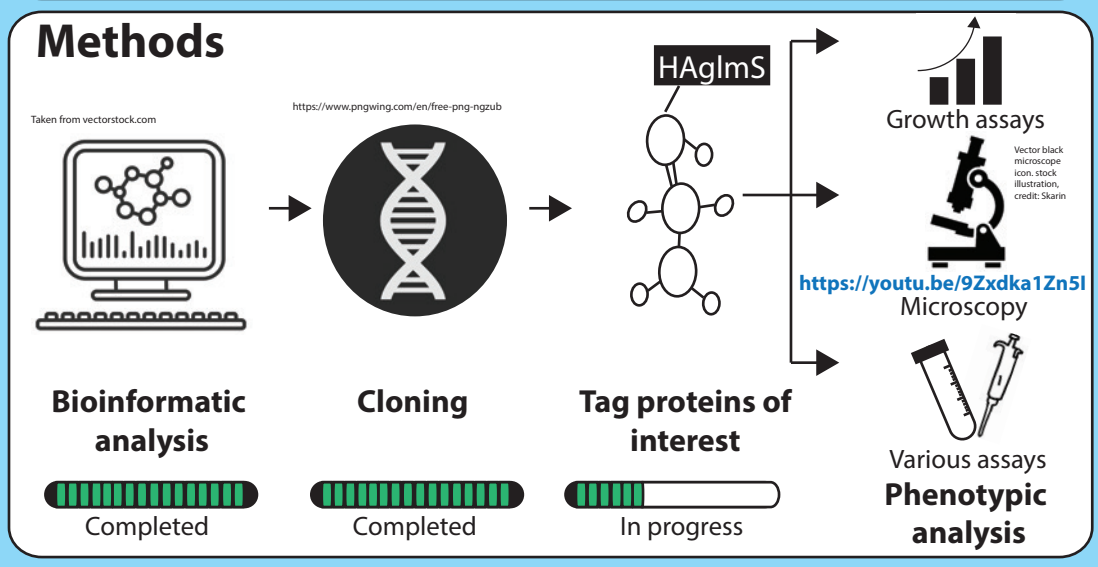
P. falciparum is responsible for the deadliest form of malaria in humans. Alarmingly the parasite has developed resistance to existing malaria drugs and there is an urgent need to identify new drugs and parasite drug targets. *P. falciparum* exports hundreds of parasite effector proteins during its blood stage to establish vital host cell modifications. **These expoted proteins: (1) establish a trafficking system to deliver other exported proteins within red blood cell (RBC), (2) make the RBC more rigid to handle parasite growth, (3) make the RBC more permeable to allow import of essential nutrients and (4) make the RBC adhesive to escape the human immune system**[1] (numbers shown on figure below). It has been previously estimated that 20% of exported proteins are essential for parasite survival during the blood stage[2], but majority of these proteins have not been characterised or experimentally verified.

Research Aim

1. Identify essential exported proteins in the blood stage of *P. falciparum* ✔
2. Characterise a subset of exported proteins identified as essential



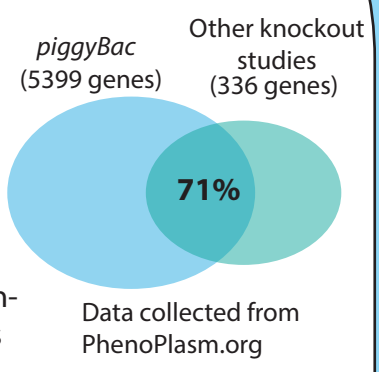
Methods

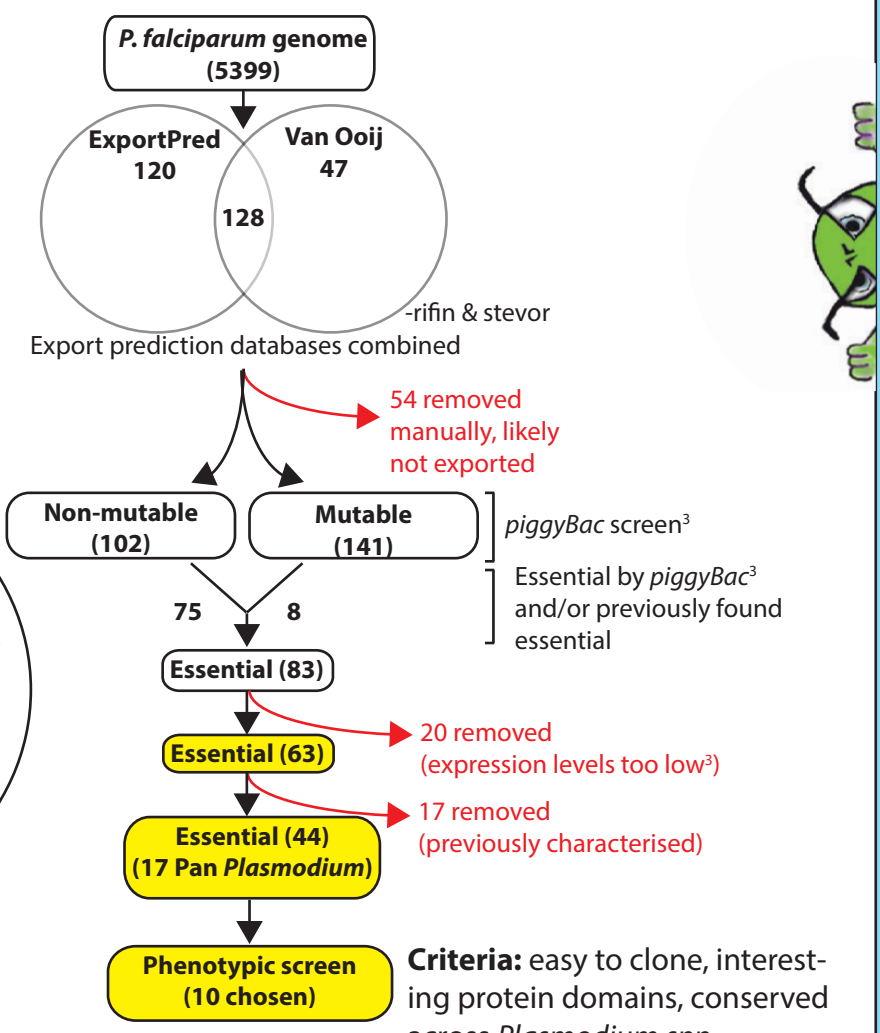


Results

1. Bioinformatic analysis

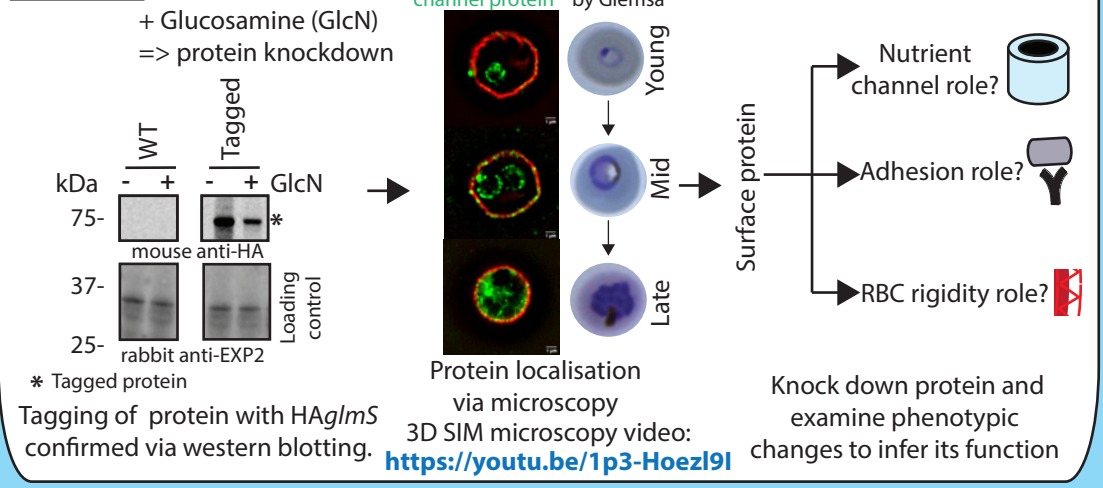
Large-scale gene knockout studies can be a good starting point of identifying essential genes, such as the *piggyBac* screen[3]. However these studies often contain a higher error rate, emphasising the need to experimentally verify these proteins. Here I used the data generated by *piggyBac* and previous knockout studies to identify exported proteins likely to be essential. Previously published export prediction databases were combined to identify exported proteins[4,5,6].





2. Phenotypic screen (Plan!)

Below is the 'typical' workflow for phenotypic screening.



Summary

- *piggyBac* (large-scale) vs previous knockout studies only show 71% agreement
- 26% of exported proteins analysed in this study were deemed essential
- Around 8% of the exported proteins were non-essential (mutable) according to *Piggybac* but essential from previous knockout studies
- 70% (44) of the essential proteins have not been previously characterised

References: 1. de Koning-Ward, T.F., *et al*. (2016). *Plasmodium* species: master renovators of their host cells. Nat Rev Microbiol 14, 494-507. **2.** Maier, A.G. *et al*. (2008). Exported proteins required for virulence and rigidity of *Plasmodium falciparum*-infected human erythrocytes. Cell 134, 48-61. **3.** Zhang, M. *et al*. (2018). Uncovering the essential genes of the human malaria parasite *Plasmodium falciparum* by saturation mutagenesis. Science 360. **4.** Sargeant, T.J. *et al* (2006). Lineage-specific expansion of proteins exported to erythrocytes in malaria parasites. Genome Biol 7, R12. **5.** van Ooij, C. *et al*. (2008). The malaria secretome: from algorithms to essential function in blood stage infection. PLoS Pathog 4, e1000084. **6.** Boddey, J.A. *et al.* (2013). Role of plasmepsin V in export of diverse protein families from the *Plasmodium falciparum* exportome. Traffic 14, 532-550.