Dissecting changes in *Plasmodium berghei* gene expression at the initial stages of mosquito midgut transmission

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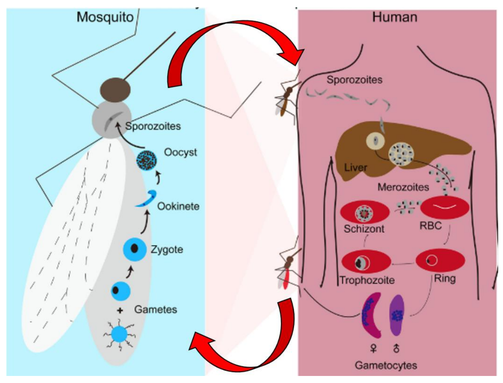
1. Background

i. Malaria, a devastating disease, is caused by the unicellular protozoan parasite, ***Plasmodium.***

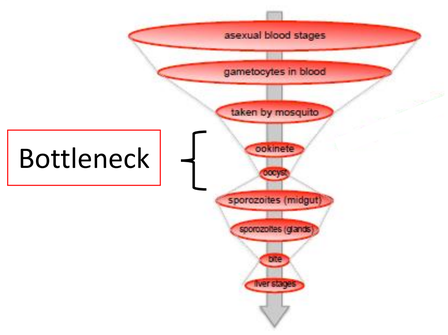
ii. The parasite has a **complex life cycle** with > 10 different stages transitioning between **asexual replication** within a mammalian host and **sexual development** in the female *Anopheles* mosquito[1].



***Plasmodium* life cycle[2]**

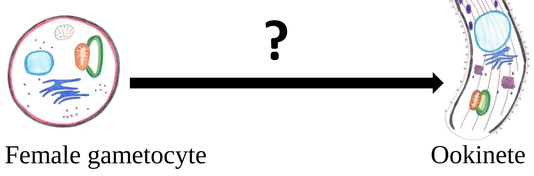
iii. These stages are defined by **distinct morphologies, metabolism and environments** which requires tight regulation by **stage-specific transcription factors**[3].

iv. Mosquito midgut stages are promising targets to **block transmission** due to a parasite load **bottleneck**[4].



v. Identifying **protein targets** requires in depth knowledge of gene regulation.

vi. **Large transcriptional changes** have been observed in the gametocyte and ookinete[5,6,7], yet the **temporal resolution** during this transition is **poorly understood**.

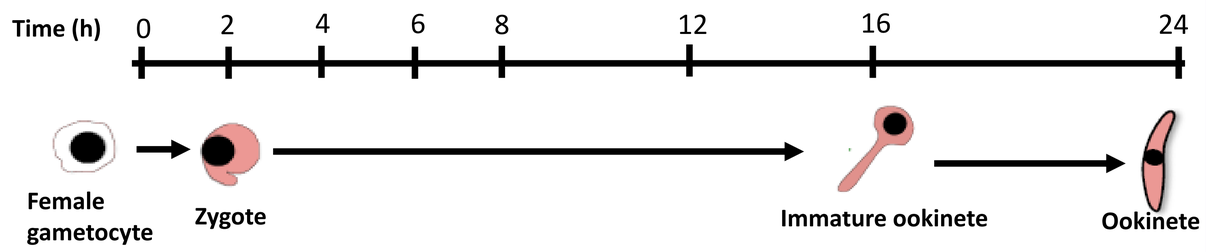


vii. We sought to elucidate transcriptional dynamics during midgut development at an enhanced temporal resolution.

2. Methods

Analysed bulk **RNA-seq** libraries at **8** time points during *Plasmodium berghei* (rodent malaria model) development in the **mosquito midgut**

4 transgenic parasite lines which do **not affect life cycle** progression[8,9,10].



Bioinformatics workflow

Read alignment ← Read quality control → Raw gene read counts → MDS plot → Differential gene expression analysis → Heatmap & clustering; GO term enrichment; Developmental genes expression plots

3.1 Results

- The parasite **transcriptome changes** throughout ookinete development.
- **2** major **shifts** in the transcriptome are observed: one immediately **after fertilisation (2hr)** and another at **≈12hr**.

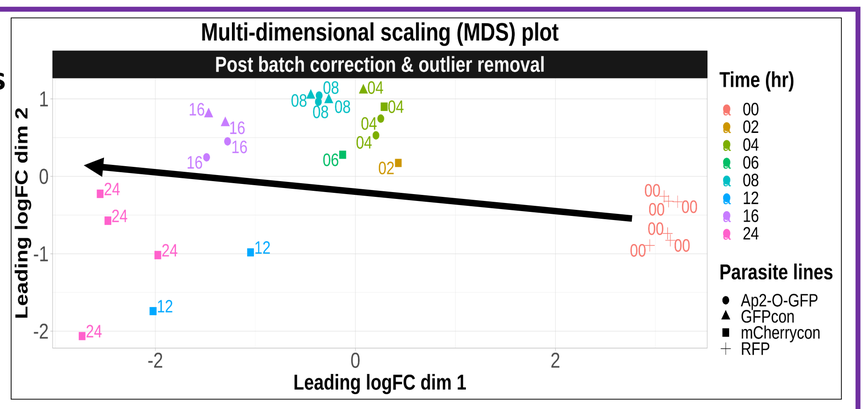


Figure 1: MDS plot with libraries clustering according to time. Black arrow = increasing temporal gradient.

- **8** gene clusters with **unique expression** profiles were identified to elucidate **biologically significant genes** that may be co-regulated.

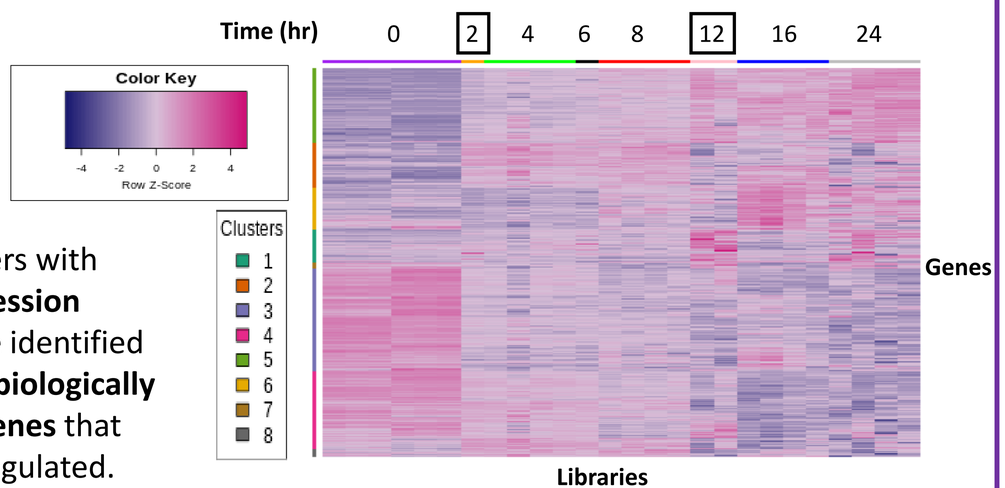
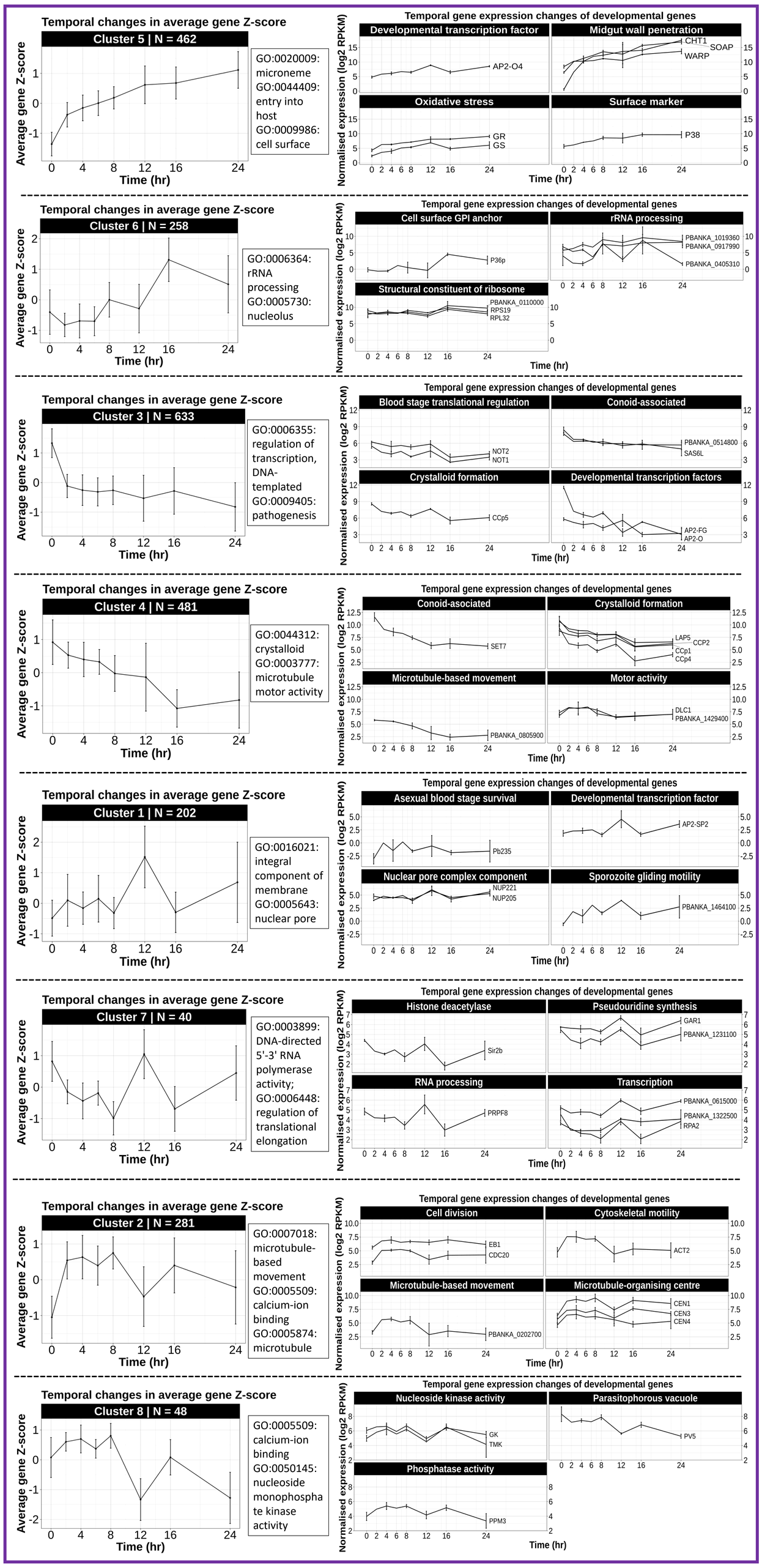


Figure 2: Gene Z-score for differentially expressed genes with false discovery rate < 0.01 at different time points during mosquito midgut development. Black boxes indicate time points at which major transcriptome shifts are observed.

3.2 Results



4. Conclusion & future work

- Mosquito stages of the *Plasmodium* parasite are promising targets to **block** malaria **transmission**. However, identifying targets for vaccines is needed which requires insight into the **gene regulatory networks** at these stages which is **poorly understood**.
- We have shown that **changes in gene expression** are observed **immediately** post-fertilisation and identified **8 gene clusters** with **distinct expression patterns**. Integration of these data with existing genomic accessibility data and transcription factor occupancy profiles will further elucidate gene regulatory dynamics at the initial mosquito midgut transmission stages.