The Role of K13 in Artemisinin Resistance

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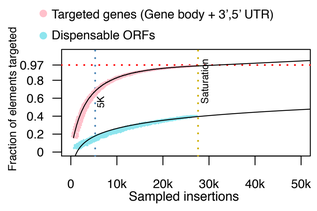
Introduction

Plasmodium *falciparum* is evolving resistance to Artemisinin Combination Therapy (ACT) in South East Asia and if resistance spreads further millions of lives will be at risk. The gene with the strongest association with resistance is K13. K13 is an ortholog of the well characterized transcriptional regulator Keap1. In this work we transcriptionally characterized a mutant with a transposon inserted in the K13 promoter region which results in dysregulation of K13 at 2 points of the intraerythrocytic cycle of the life-cycle to identify the processes regulated by K13.

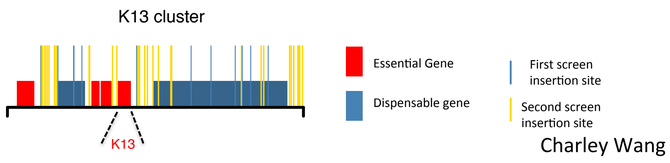
Methods

K13 is Essential



'Dispensable ORFs' mean the ORF targeted by sampled insertions

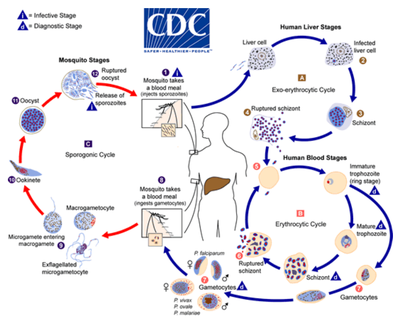
'Targeted genes' indicates the 'gene body + 5_3 UTR' targeted by sampled insertions

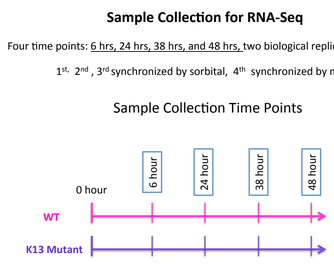


Charley Wang

K13 cannot be knocked out, suggesting regulation is the only way to study K13's function. Isogenic mutants were created from a P. *falciparum* lab strain and despite creating enough mutants to have disrupted every non-essential gene in the genome K13 was not knocked out. A mutant was created with an insertion in the 5' upstream region of K13 resulting in a strain that is more sensitive to artemisinins.

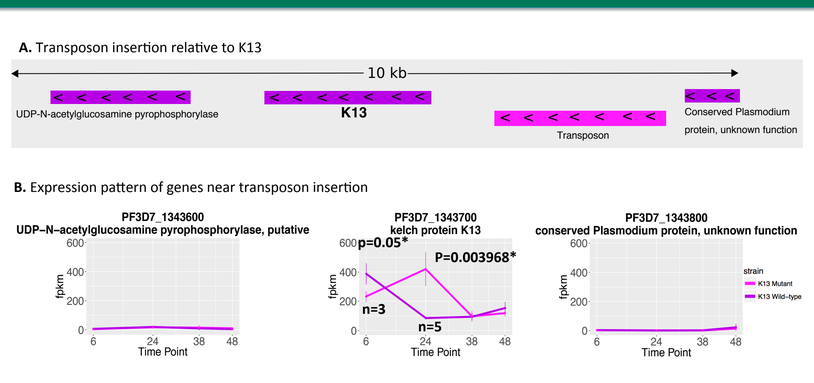
Study Design



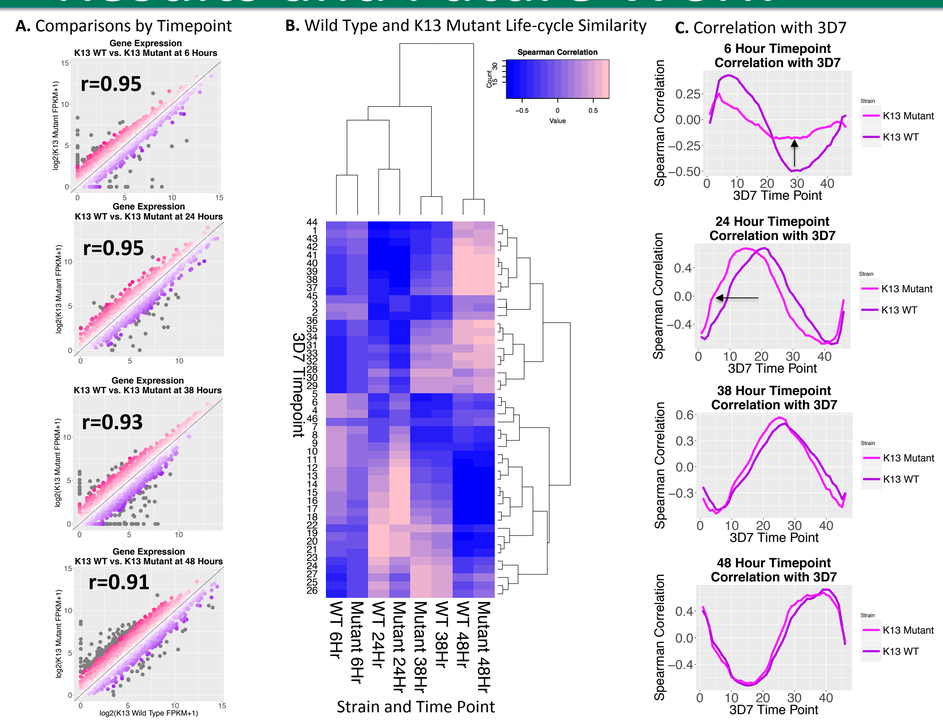


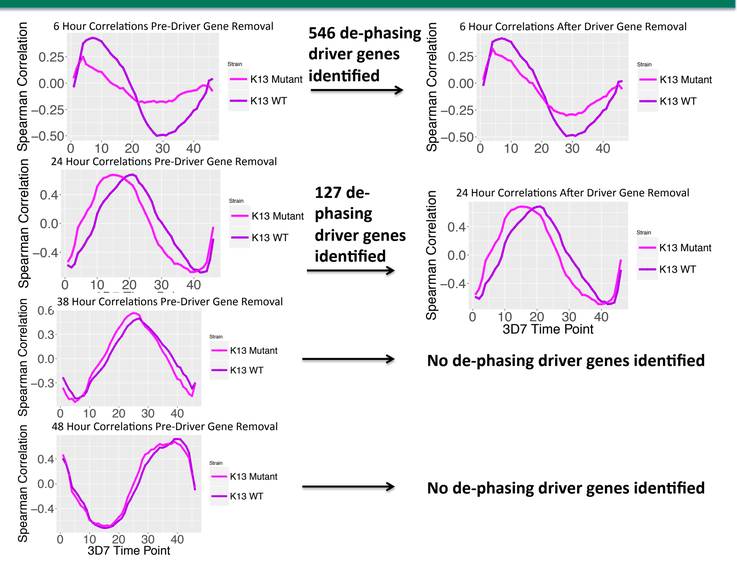
P. *falciparum* has a complex life cycle. Gene expression during the erythrocytic cycle was studied because this is the part of the life cycle that causes disease. The mutant and the parent strain were both synchronized and RNA was harvested at 4 time points representative of the major stages of the erythrocytic cycle. The RNA was sequenced on an Illumina Miseq and the resulting reads aligned to a reference P. *falciparum* genome using HISAT2 and FPKM values obtained using Cufflinks and Cuffnorm. Changes in the expression of gene sets were evaluated using GSAR and GAGE.

Results and Future Work



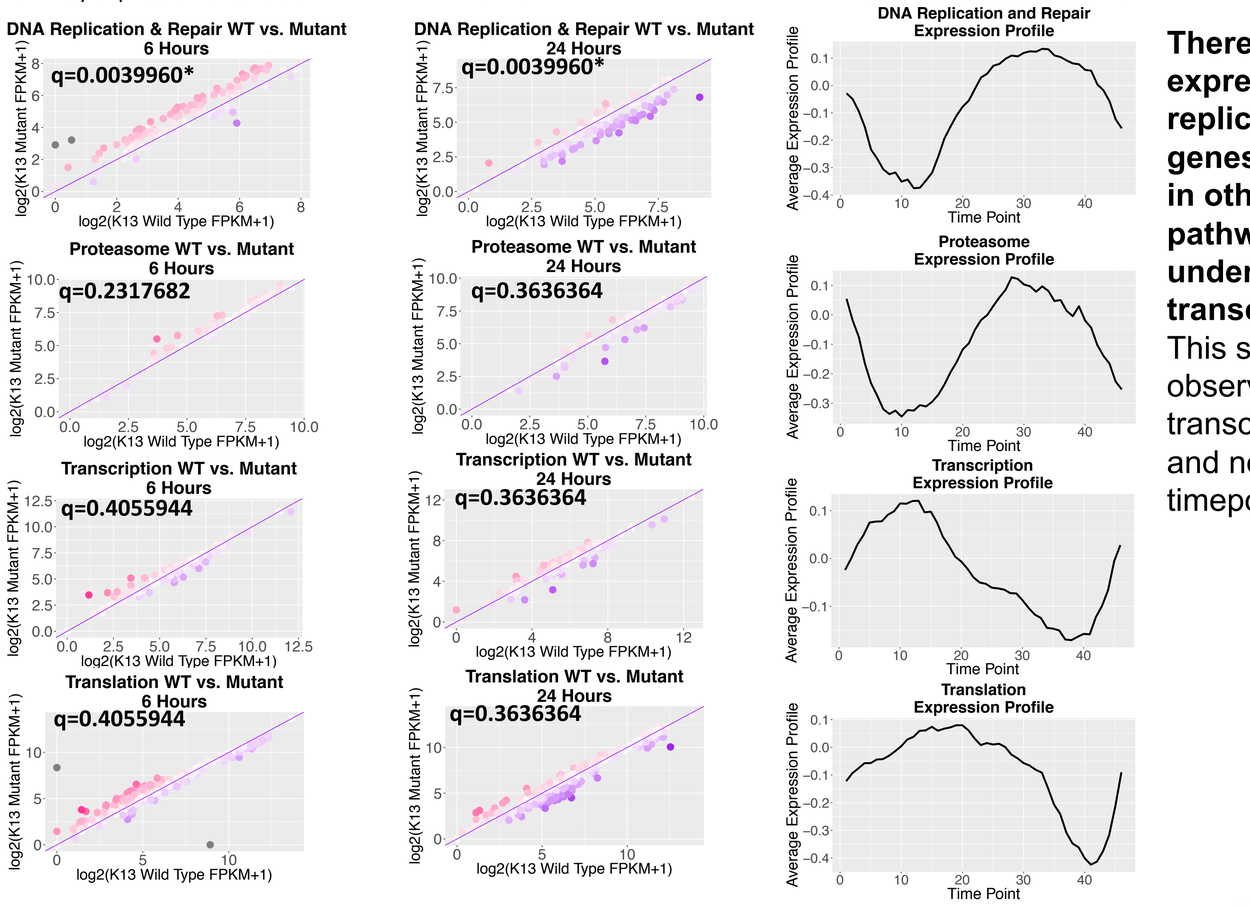
The RNA-seq data confirmed that the K13 mutant has aberrant down-regulation of K13 at the beginning and of the disease causing erythrocytic cycle and up-regulation at the midpoint. The data also showed that the expression levels of K13's neighbors are unaffected by the insertion indicating the only difference between the wild-type and mutant strain is K13 dysregulation.



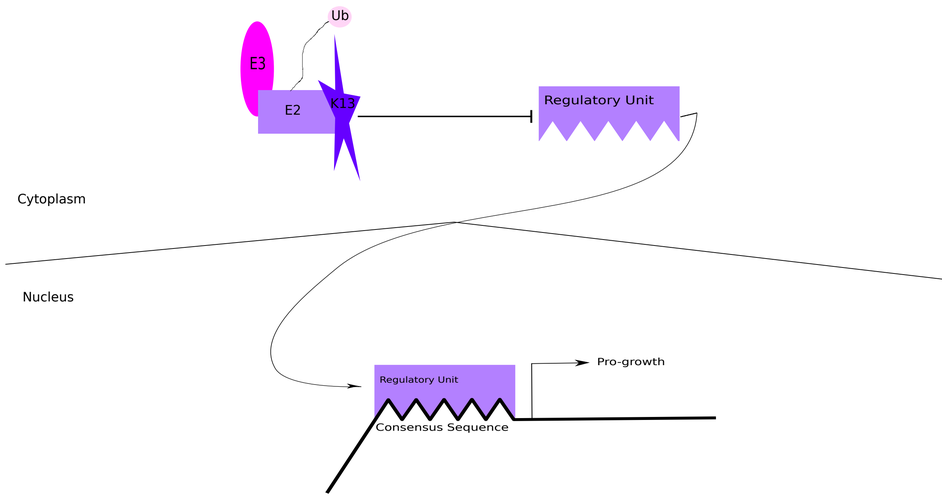


The mutation does not cause major disruptions to the transcriptome or progression through the life-cycle in the mutant expect the 6 hour timepoint shows a transcriptomic shift towards latter timepoints.

An algorithm was developed to identify the genes most responsible for the observed transcriptome shift. DNA replication and repair genes were over-represented amongst these de-phasing driver genes. The mutant maintaining a late stage transcriptome longer is the likely explanation for the mutants observed increased sensitivity to arteminsins.



There is a shift in the expression of DNA replication and repair genes that is not present in other housekeeping pathways that are also under similar rates of transcriptional regulation. This suggests that the observed changes in the transcriptome are specific and not the result of timepoint sampling error.



The data and K13's similarity to the E3 ubiquitin substrate adapter Keap1 suggest that K13 acts as a negative regulator of a pro-growth regulatory unit. Further work is needed to identify the pro-growth regulatory unit.