Single-cell RNA-seq of *Plasmodium chabaudi* parasites reveals transcriptional changes underlying malaria virulence, circadian rhythms, and transmission potential.

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Plasmodium parasites, the causative agents of malaria, are characterised by synchronous bursting of infected red blood cells during the asexual life cycle in the vertebrate host and sexual development within the mosquito host. However, the severity of disease, robustness of host immune response, and mosquito transmissibility differs between *Plasmodium* species and strains. Experimental infections with rodent malaria parasites (*P. berghei, P. yoelii, P. chabaudi,* and *P. vinckei*) have contributed significantly to our understanding of the molecular mechanisms underlying malaria disease heterogeneity. *P. chabaudi* in particular has proven to be an invaluable tool in the study of strain-specific virulence and drug resistance, owing to the remarkable genetic diversity between strains of the same subspecies, and the isolation of parasite lines that differ dramatically in disease severity.

In the present study we provide, for the first time, complete single-cell transcriptomes for the intraerythrocytic life cycles of three genetically distinct *P. chabaudi* strains: (i) *P.c. chabaudi* AS, (ii) *P.c. chabaudi* AJ, and (iii) *P. chabaudi* subsp. *esekanensis*. Both *P.c. chabaudi* strains AS and AJ were isolated from the same African thicket rat (*Thamnomys rutilans*) in 1969 in the Central African Republic and differ in their growth rates, 24 h circadian rhythm synchronicity, and host virulence. The independent clonal line, *P. chabaudi* subsp. *esekanensis*, was isolated from a *Hylomyscus* sp. mouse in Cameroon in 1973 and has recently gained renewed interest because of its noticeably greater propensity to form sexual-stage parasites in peripheral blood, a sign of increased transmissibility to mosquitoes downstream.

Comparative analyses of these data reveal both shared and divergent cell populations and developmental trajectories between all three *P. chabaudi* lines. Known *Plasmodium* stage-specific genetic markers were used to define the asexual life cycle trajectory from ring to schizont stage as well as immature and mature sexual-stage gametocyte populations. Notably, differential DNA replication dynamics, evidenced by strain-specific merozoite-per-schizont numbers and growth rates, were reflected in single-cell expression data. These new single-cell atlases, coupled with matched phenotypic data, provide new insights into malaria parasite biology, with implications for functional studies and the discovery of antimalarial drug/vaccine targets in future.