How do nucleotide-limiting drugs affect the cell cycle of *Plasmodium falciparum*?

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P. falciparum replicates by schizogony, an unusual non-binary-fission type of cell cycle. Schizogony involves an asynchronous replicative process by which an average of 20 nuclei are produced from a single parasite, which then undergoes a singular cytokinesis event. *Plasmodium* cell cycle control is poorly understood, as the parasite appears to lack the common checkpoint kinases, cyclins and cyclin dependent-kinases (CDKs) found in other eukaryotes, which control entry to subsequent cell cycle stages. The parasite also undergoes multiple, asynchronous rounds of S phase and karyokinesis before cytokinesis, and hence appears to lack a canonical G2 phase.

Previous studies using live cell microscopy as well as labelling of nascently replicated DNA with thymidine analogues have suggested the existence of a limiting factor preventing unlimited simultaneous rounds of genome replication. One potential limiting factor is the availability of a nucleotide pool. To investigate this, two drugs, hydroxyurea and pyrimethamine, which target nucleotide biosynthesis via differing pathways, were used to lower the available nucleotide pool. Pyrimethamine; an antifolate drug which target the DHFR enzyme of the parasite folate pathway is used as intermittent preventative treatment in pregnancy (IPT) in combination with sulfadoxine, another anti-folate. Hydroxyurea on the other hand, targets the ribonucleotide reductase enzyme which is involved in conversion of ribonucleotides into deoxyribonucleotides. This drug is not used directly as a malaria treatment but research looking at its use as a sickle cell anaemia (SCA) drug found that when examining malaria as a common secondary co-existing condition, its incidence decrease in the months following hydroxyurea treatment,

The subsequent parasite responses to these drugs were tracked throughout the erythrocytic cell cycle via time-course brightfield microscopy counts, supported by flow cytometry. Additionally, immunofluorescence assays were performed to track merozoite numbers per schizont, and the timings of the first nuclear replication (1n-2n). We found that depletion of the nucleotide pool led to slower lifecycle progression, a delay in the first nuclear replication, decreased numbers of nuclei per schizont, and subsequent reduced parasitaemia after reinvasion. Overall, these results suggest that nucleotide supply is indeed a limiting factor in the progress of schizogony. Greater understanding of *Plasmodium* replication and how it is controlled is vastly important, because current and historical antimalarial drugs (such as pyrimethamine) target the replication of the parasite's genome, and because this unique cell cycle could still harbour further novel drug targets.