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Determining whether DNA replication is affected by the pilE sRNA and G4-structures to initiate pilin antigenic variation

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### Background

*N. gonorrhoeae* uses a complex diversity generation system to produce many pilE and pilin variants, leading to host immune response evasion. We have postulated that pilin antigenic variation initiates when chromosomal replication is affected by the sRNA-R-loop and G4 structures.

## Aim/Methods

Three gonococcal strains (FA 1090 parent strain, sRNA- and G4 mutants) were grown in liquid medium and replication initiation was blocked by inhibiting translation with tetracycline. After removal of tetracycline, replication restarts. To analyze the replication fork progression, genomic DNA was isolated at various times and gene density determined by Nanopore sequencing.

## Results

Preliminary results show that the genome coverage was lower at the pilE gene in the FA1090 parent strain (Mean = 0.78, SD = 0.07) and the G4-mutant (Mean = 0.91, SD = 0.09) than the sRNA-mutant (Mean = 1.03, SD = 0.02). Significantly lowest genome coverage was at the sRNA and G4 structure in the FA1090 parent (Mean = 0.68, SD = 0.02) and G4-mutant (Mean = 0.68, SD = 0.03) than sRNA-mutant (Mean = 1.01, SD = 0.01, p-value = < 0.0001).

## Conclusions

Since both the pilE sRNA-R-loop and G4 structures are both known to interrupt with replication, these preliminary results suggest that these structures act to interrupt replication as a way to initiate the gene conversion reactions. We are investigating whether interrupting replication initiation using a conditional DnaA strain and by localizing components of the replication complex can provide a more detailed view of the interactions between DNA replication and the sRNA-R-loop, and G4 in initiating pilin antigenic variation.