Analyzing the Function of NucS in *M. smegmatis*

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Research Question

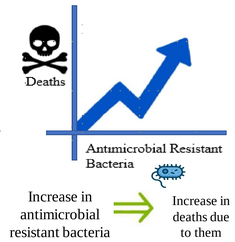
This project aims to determine the function of the five amino acid sequence at the C-terminal of NucS and to investigate the interaction between NucS and DnaN.

Hypothesis

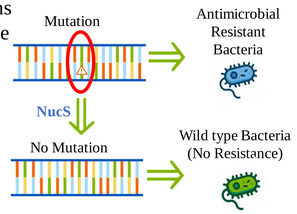
We hypothesize that the five amino acids at the C-terminal of NucS aid NucS in binding to DnaN during DNA mismatch repair.

Background

Antimicrobials are critical in the treatment of bacterial infections and thus the preservation of global health. However, many bacteria can develop antimicrobial resistance (AMR) (Davies & Davies, 2010). AMR can be conferred in many ways, though the most common method involves mutations during DNA replication (Annunziato, 2019).



The increase in AMR poses a major risk to global health, with death rates caused by AMR predicted to skyrocket to 10 million deaths annually within the next three decades (Stéphane Dujarric, 2019).



NucS has been shown to prevent mutations in *Mycobacteria*, a genus which includes *Mycobacterium tuberculosis.* More understanding of its precise function may elucidate further directions for protecting and promoting DNA repair in *Mycobacteria*.

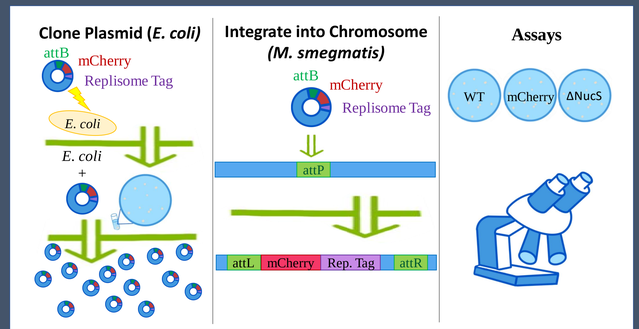
Investigating the interactions of NucS with other proteins in the repair pathway is one such inquiry that would shed light on its function (Cebrián-Sastre et al., 2021). Such interactions may be observed by tagging NucS.

Main Takeaway

This project involved the construction of a plasmid used to tag NucS at its C-terminus. The tag used was the fluorescent protein mCherry. After tagging of *M. smegmatis nucS*, the cells were observed with fluorescence microscopy and tested for a mutagenic phenotype.

Methods

- Ligate mCherry fragment into ORBIT integration plasmid
- Electroporate ligation into *E. coli* and grow on LB chloramphenicol plates
- Integrate into chromosome of *M. smegmatis* using ORBIT
- Determine frequency of spontaneous mutation with rifampicin plates
- Observe cells containing NucS-mCherry fusion under fluorescence microscope



Results

Figure 1
PCRed junctions from chromosome of *M. smegmatis* after integration of the plasmid. All three samples contained the correct sequence.

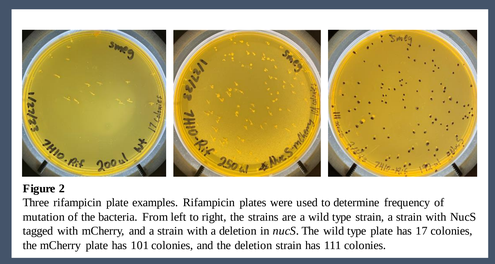


Figure 2
Three rifampicin plate examples. Rifampicin plates were used to determine frequency of mutation of the bacteria. From left to right, the strains are a wild type strain, a strain with NucS tagged with mCherry, and a strain with a deletion in *nucS*. The wild type plate has 17 colonies, the mCherry plate has 101 colonies, and the deletion strain has 111 colonies.

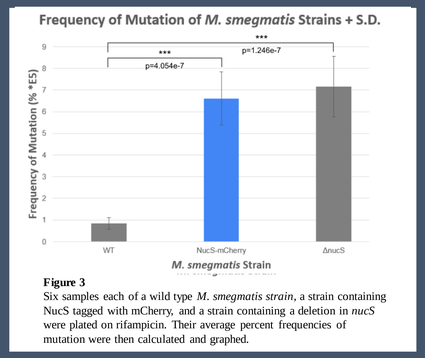


Figure 3
Six samples each of a wild type *M. smegmatis strain*, a strain containing NucS tagged with mCherry, and a strain containing a deletion in *nucS* were plated on rifampicin. Their average percent frequencies of mutation were then calculated and graphed.

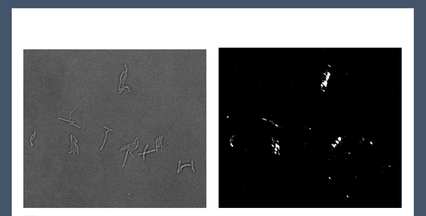


Figure 4
Images taken with a fluorescence microscope of one sample of *M. smegmatis* containing NucS tagged with mCherry. The image on the left focuses on the cells, while the image on the right indicates the foci in each cell. Some cells have no foci, some have one to two foci, and others appear to have multiple foci.

Analysis

As indicated by our graph, there was a significant difference between the frequency of mutation of our wild type and our NucS-mCherry strains, but not between our ΔNucS and NucS-mCherry strains, indicating that our mCherry tag interfered with the ability of NucS to repair mutations. This result suggests that the five C-terminal amino acids of NucS may be necessary for functions of NucS other than binding, or that additional amino acids may be necessary for the binding of Nucs to the replisome.

We cannot draw conclusions on the interaction between NucS and DnaN because our tagged NucS was unable to repair DNA mismatches and may not have accurately reflected the interaction of wild type NucS with DnaN.

Future Work

- Combine our mCherry-tagged NucS with a strain containing GFP-tagged DnaN
- Tag NucS at its N-terminus
- Locate NucS at different stages of the cell
- Further identify other critical amino acids in NucS

Conclusion

As mutations are a key cause of antimicrobial resistance in bacteria, learning more about proteins such as NucS which prevent mutations may open new paths to research that addresses antimicrobial resistance.

This project constructed a plasmid to investigate the function of the replisome tag of NucS and found that separating it from the rest of the protein caused increased mutagenicity.

Further research may involve tagging NucS at its N-terminus and observing NucS tagged with mCherry in a cell expressing DnaN tagged with GFP.