Rev1 Protein Purification

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What is Rev1?

- **Hypothesis**- R324 and L325 side chains are key to evicting the DNA template base [1]
- **Ultimate goal**- use our custom tweezers to study DNA replication
- **Current Goal**- X-ray crystallographic studies of R324G/L325G Rev1 to examine the mechanism by which Rev1 evicts the DNA template base

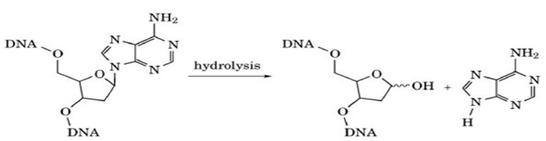
Cancer Research

- Replication of damaged DNA by Classical DNA Polymerases leads to genetic mutations
- Genetic mutations lead to disorders and cancer
- Rev1 is a Y-family DNA polymerase with a unique mechanism
- Catalyzes non-mutagenic replication of abasic sites and exocyclic guanine adducts
- DNA Template base is evicted from active site
- Replaced with Arginine from Rev1

DNA Damage

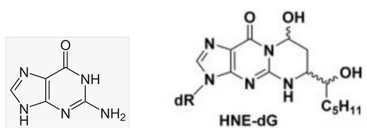
Abasic Sites

- Formed by hydrolysis at physiological pH

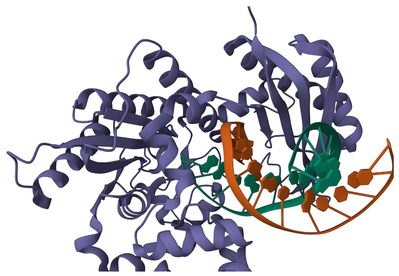


Exocyclic Guanine Adducts

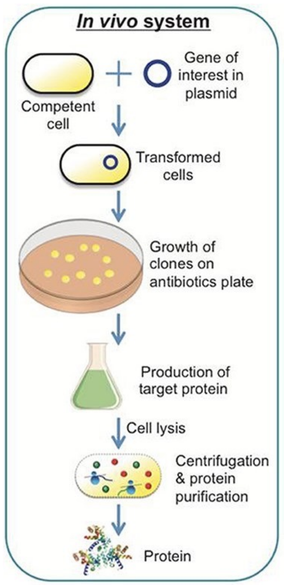
- Extra rings to nucleotide bases via carcinogens
- Alter Watson-Crick binding structure and coding of DNA

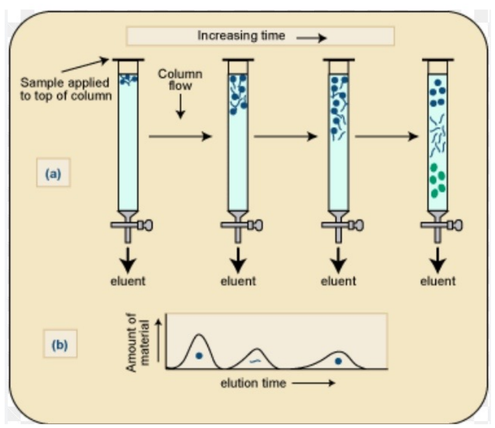


Rev1



Protein Purification





Initial Results and Current Progress

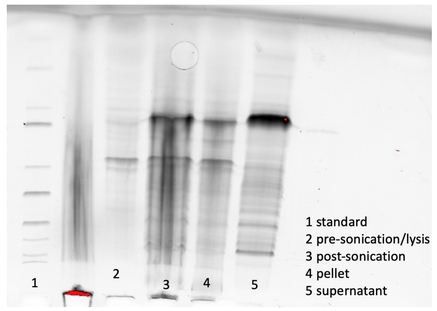


Figure 1:SDS-PAGE Wild Type Rev1

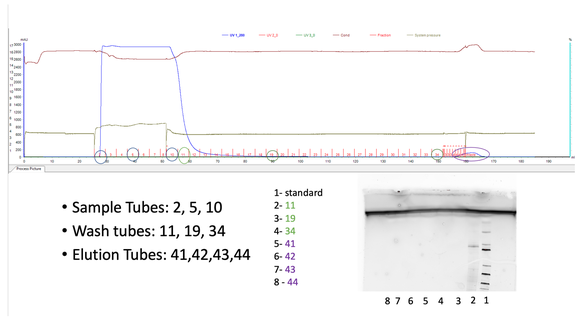
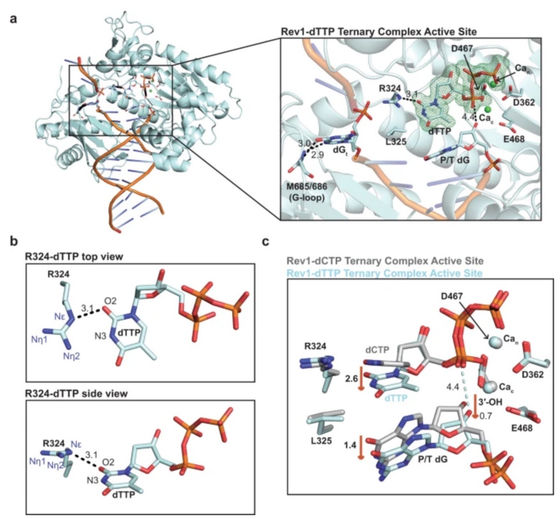


Figure 2:GS Trap Elution Data



Rev1/DNA/dTTP ternary complex [1]

Future Plans

- Wild Type Purification
- Mutant Purification
- X-Ray Crystallography
- Integration with Optical Tweezers Project

References

[1] Tyler M Weaver, Timothy H Click, Thu H Khoang, M Todd Washington, Pratul K Agarwal, and Bret D Freudenthal. Mechanism of nucleotide discrimination by the translesion synthesis polymerase rev1. *Nature Communications*, 13(1):2876, 2022.

Figure 3:Weekly Meeting Progress

Acknowledgements

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