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Background

Pentavalent chromium has been shown to create DNA damage by oxidizing the base guanine to two unusual hydantoin moieties: Sp and Gh. These lesions cause G→T transversion mutations and can be recognized by the BER enzyme Nei. Because of its structure and proximity to Nei in the genome, we hypothesize that YbgL, an uncharacterized *E. coli* protein, recognizes the Sp and Gh lesions.

YbgL is a member of the Lamb/Ycsf superfamily of proteins. This family is named because of its homology to LamA which hydrolyzes cyclic lactam molecules such as 2-pyrrolidone moieties (Katz et al. 1989). Due to the hydantoin chemical scaffold of the chromium –induced lesions Sp and Gh, we are investigating whether YbgL (and the LamB/YcsF superfamily to which it belongs) perform similar chemistry to the hydantoinase and cyclic amidohydrolase superfamily of enzymes. We wish to investigate whether YbgL is capable of hydrolyzing hydantoin-containing DNA lesions to Gh as *E. coli* cells lacking the YbgL gene cannot make Gh endogenously nor in response to chromium treatment (Savannah Whitfield–2016 in preparation). Specifically, we wish to investigate whether YbgL is required for the initial step in the following hydrolysis of Sp (Fig. 1).

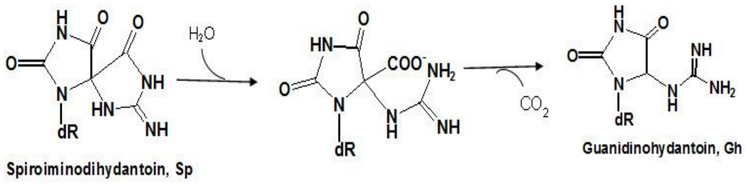

Figure 1. (above) Mechanism of oxidation from Sp to Gh.

Figure 2. (right) Cr(III)-Salen crystals, the precursor for the Cr(V) carcinogen, were synthesized according to Coggon and McPhail (1970).

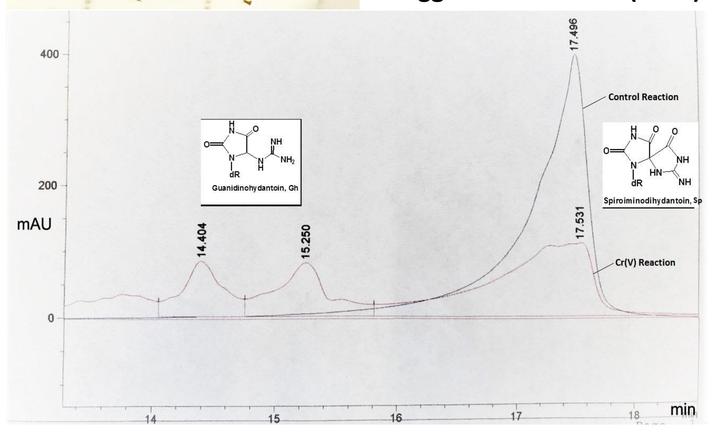

Figure 3. Chromatogram of the control and Cr(V)-reacted synthetic oligo with peaks labelled based on Sugden *et al.* (2001).

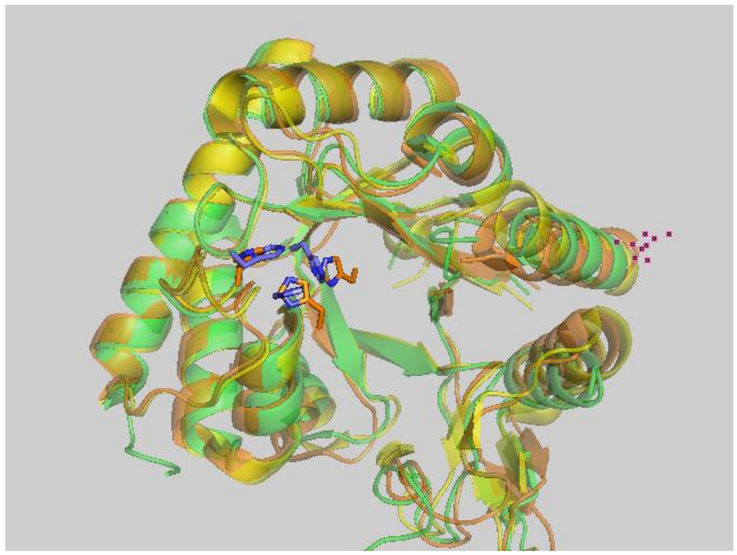

Figure 4. Above is an overlay of one subunit from each of three LamB/YcsF proteins: YbgL (green; PDB: 1xw8) and two putative lactam utilization proteins from *Pyrococcus horikoshii* (yellow; PDB: 1v6t) and *Thermos thermophiles* (orange; PDB: 2dfa). Note the histidine cluster of YbgL.

Methods

Oligo Oxidation

To test this hypothesis, we began by oxidizing 100 uM of a synthetic oligo (5'ACCAGCGXCCGCACCAGT, X is 8-oxo-G) with 20 uM Cr(V) at a pH of 8.05 in 100mM K_2HPO_4 for 30 min. at room temperature.

HPLC Protocol

The oligonucleotide oxidation products were separated using a Dionex Nucleopac PA-100 4 x 250-mm anion exchange column using 10% acetonitrile with a gradient of 10-100% 1.5 M Ammonium Acetate in 10% AcCN pH 6.01 over 20 min. (Fig. 3).

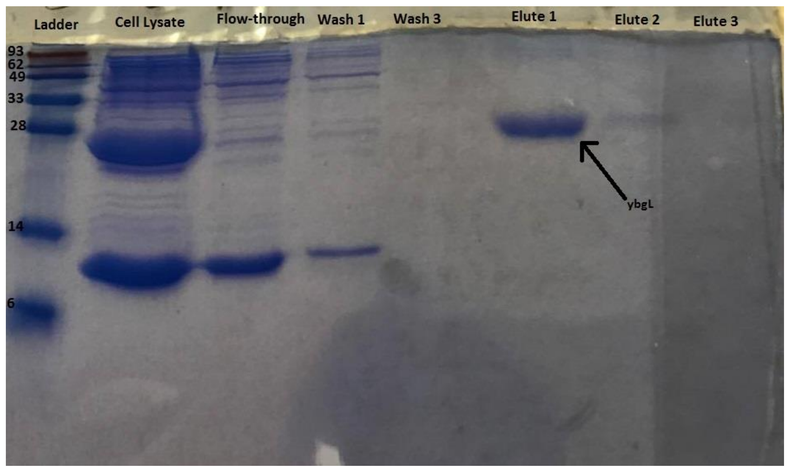

Figure 5. 15% SDS-PAGE gel of the fractions collected from Ni-NTA chromatography. The large band just below 28 kDa is YbgL, since this protein is about 26 kDa.

Ni-NTA Purification Protocol

The cell lysate was loaded onto a Ni-NTA column (Qiagen) equilibrated with NPI-10 (150 mM NaCl, 50 mM Na2HPO4, pH 7.52, 10 mM imidazole). Three washes of NPI-20 were followed by three elutions of NPI-500 (Fig. 5). Purity of YbgL was determined by 15% SDS-PAGE.

Conclusions

Oligonucleotides contain Sp or Gh were synthesized and purified. Following analysis by mass spectrometry, these will be used to measure the binding of modified oligos to YbgL. Structural modeling indicated a structural similarity of YbgL to the active site of hydantoinase molecules as well as other manganese containing enzymes (Figure 6).

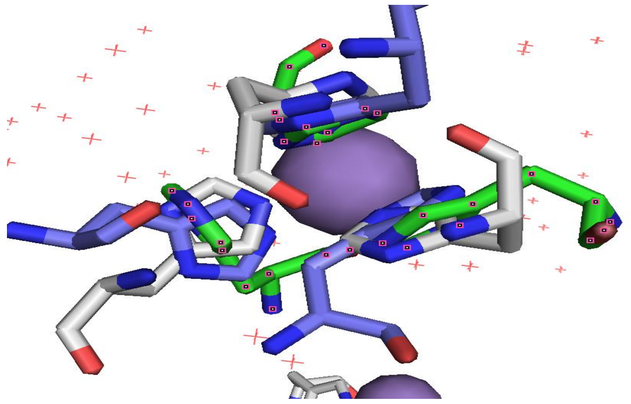

Figure 6. The tri-histidine cluster present in YbgL (H57, H106, and H3; PDB: 1xw8) mirrors those found in D-hydantoinase (PDB: 1NFG) and oxalate oxidase (PDB: 2ete) which both bind manganese cofactors.

Future Directions

The next step in this project will be to demonstrate binding of YbgL to oxidized DNA lesions *in vitro*. To provide more insight into the structure and function of YbgL x-ray crystallography studies will be conducted to determine whether the tri-histidine cluster of YbgL requires a metal cofactor to stabilize and form and active protein.

Acknowledgments

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References

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