TruD's Effect on RNA Oxidation

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Does TruD's preferential binding to oxidized RNA protect cells?

Introduction

- Oxidative damage, a result of aging, causes neurodegenerative disease by altering the cell's genetic makeup.
- TruD is an enzyme that binds oxidized RNA with high affinity. Mutants of TruD lacking catalytic activity lack the ability to preferentially bind to oxidized RNA.
- The presence of TruD allowed the cell to survive for an extended period of time compared to the time survived without TruD.
- In this proposed work, we plan to examine if TruD's binding capability to oxidized RNA is responsible for cell protection under oxidative stress. The elucidation of TruD's behavior on oxidative RNA will be examined by comparing the inactive mutants of TruD with the normal enzyme.

Method

Construction of D80T and D80N mutants:

1. Using the process Recombineer, a single nucleotide will be introduced into the genomic DNA sequence and will convert the normal TruD gene into a TruD mutant encoding an inactive protein.
2. The mutants will be selected by discriminative PCR screening reactions.
3. The use of the miniprep kits available commercially, a plasmid pET-coco1 as the vector, and the TruD genes will be synthesized by PCR using the high-fidelity Pfu polymerase and a thermocycler.
4. The TruD genes will then be cloned into pET-coco1 using appropriate restriction enzymes and DNA ligase.
5. The product is then transformed into E. coli cells and screened by digestion or DNA sequencing. This will result in clones of TruD.
6. The E. coli cells containing the wild type and mutant forms of truD will be grown in YT medium and treated with the oxidant tert-Butyl hydroperoxide (TBHP) at various concentrations. Colony forming units (cfu) will be measured by the alamar blue dye.

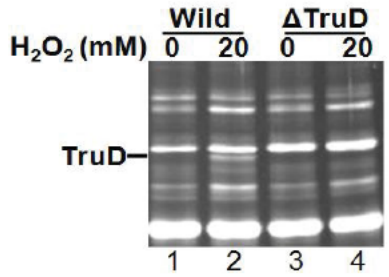

Image 1. TruD binds oxidized RNA with high affinity protein pull down experiments

Using cell extracts with normal and oxidized RNA revealed the TruD protein as one of the proteins having higher affinity to oxidized RNA but not to normal RNA.

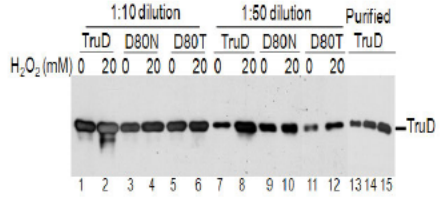

Image 2. Western blot analysis of pulled out cell lysate with anti-His-antibody

Western blot analysis with anti-His-antibody evidently shows that the protein band is TruD as expected (lanes 1-12). Purified His-tagged TruD proteins were also shown signals with anti-His-antibody, and they ran at the same level with pulled out TruD in cell lysates (lanes 13-15).

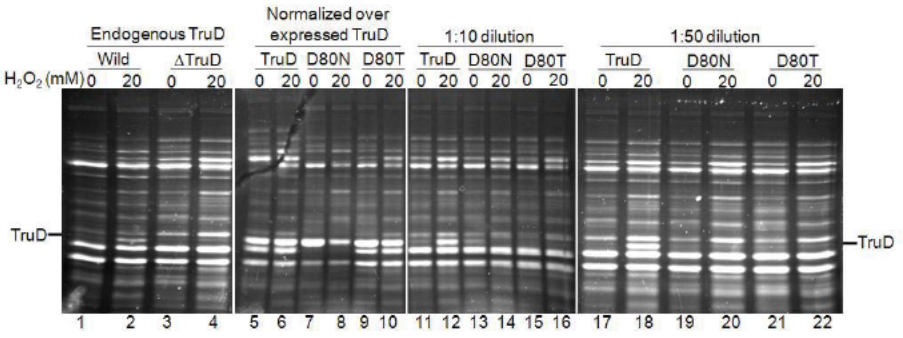

Image 3. Wild type TruD (but not the inactive mutants D80N and D80T) shows high affinity to oxidized RNA.

Replacing aspartate residue at the 80th position (D80) with asparagine (D80N) or threonine (D80T) resulted in complete loss of catalytic activity.

Anticipated Results

1. If the mutants do not protect the cell from oxidative stress treatment, then the catalytic activity and binding to oxidized RNA are important for cell protection. This will lead to the study of how the catalytic activity and binding of oxidized RNA would be involved in reducing RNA oxidation and protects cells.

1. If the mutants protect the cell against oxidative treatment in the same way as the wild type TruD does, then it will be clear that the protective role of TruD is independent of its catalytic activity. This would prompt us to study other domains of the TruD protein for controlling RNA damage and protecting cells against oxidative stress.

Discussion

In summary, TruD binds oxidized RNA with high affinity. The enzyme also was proven to require aspartate residue at the 80th position in order to maintain catalytic activity. The role of TruD in protecting oxidative stress will be elucidating using a growth experiment. This experiment will compare the effect of cell survivability under oxidative stress with TruD and its mutants. If TruD protects cells under oxidative stress, it is possible to ultimately reduce the chance of neurodegenerative diseases from occurring.

References

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