Poster Presentation P8

# STABILIZING A [4FE-4S] CLUSTER TO OXYGEN: EFFECTS OF AMINO ACID SUBSTITUTIONS NEAR THE CYSTEINE LIGANDS IN THE FNR TRANSCRIPTION FACTOR

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The FNR protein is an oxygen-sensing transcription factor found in the facultative anaerobe Escherichia coli. Dimerization and DNA binding of wild type FNR are coupled to the incorporation of a [4Fe-4S] cluster, which is ligated by cysteine residues at positions 20, 23, 29, and 122.

In the presence of molecular oxygen, this cluster degrades causing a conformational change that renders the protein inactive. Previous research has shown that the Leu28His mutant FNR protein has an oxygen stable [4Fe-4S] cluster. Several more amino acid substitutions have been made at residues adjacent to the cysteine ligands. The in vivo activity of these mutant FNR proteins has been evaluated by beta-galactosidase assays under aerobic and anaerobic conditions. Most of the mutant proteins retained similar activity to that of the wild type protein, with significant activity under anaerobic condition and little activity under aerobic conditions. However, replacement of the Leu28 with Lys or Arg resulted in proteins that are active under aerobic conditions.

These results suggest that a basic amino acid residue at position 28 stabilizes the cluster to oxygen. The Leu28Lys FNR protein has been isolated and preliminary characterization of its [4Fe-4S] cluster by absorption spectroscopy shows that like the Leu28His FNR protein, the [4Fe-4S] cluster in Leu28Lys FNR is stable in the presence of oxygen.

The [4Fe-4S] cluster of the Leu28Lys mutant protein is currently being further characterized in order to give a better understanding of how a basic amino acid at position 28 helps to stabilize the cluster in the presence of oxygen.