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The effect of PEPCK if the the transcription fact dFOXO is knocked down

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The Effect On PEPCK If The Transcription Factor dFOXO Is Knocked Down

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Introduction

- PEPCK stands for Phosphoenolpyruvate carboxykinase, this enzyme typically is a key enzyme for the liver and the kidney however this gene has also been located and seen in the small intestine and colon. PEPCK helps with this metabolic pathway by converting oxaloacetate into phosphoenolpyruvate and carbon dioxide (O'Brien et al 1990).
- FOXO is also very interesting due to their ability to play a role in death and longevity, this means that FOXO not only helps with inducing stress levels but when conditions get really extreme FOXO might lead to cell death.There are four of these FOXO transcription factors in mammals, they are called FOXO1, FOXO6, FOXO3, and FOXO4. When these transcription factors are binded to the target gene they begin to affect the development and metabolism (Jiramongkol and Lam 2020).

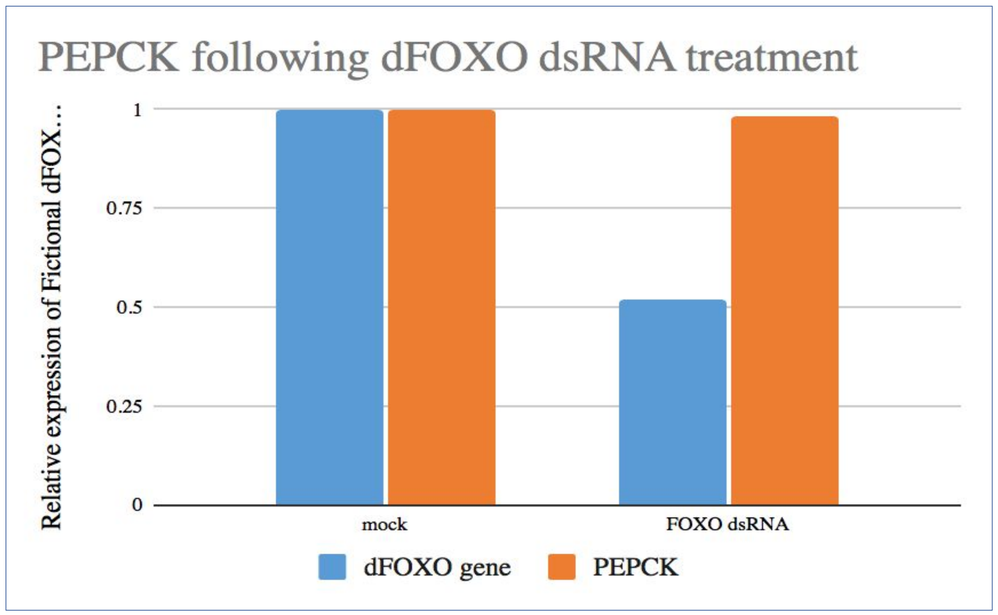
Methods

- The experiment was first started by thawing out the chemically competent cells that were on ice.
- Cultured bacterial cells were harvested by adding 1.5 mL of cultured cells to a centrifuge tube then spun in the centrifuge for 1 minute then the supernatant was discarded.
- PCR was completed the sample was diluted and cleaned.
- The concentrated eluted DNA was mixed with loading dye and 5 microliters of the sample was added to the agarose gel and electrophoresis was conducted f
- In vitro transcription for each T3 RNAP and T7 RNAP, by thawing frozen reagents on ice then vertexing the 10x Reaction Buffer and NTPs.
- The final wash 2.5 mL of serum free media was added and dsRNA was also added and incubated after for 1 hour at room temperature. 5 mL of complete media was then harvested and assayed.

Results

- The relative expression of dFOXO is 0.521 after the treatment and the PEPCK remained around 1.
- According to the graph the knocking down of dFOXO resulted in the gene expression decreasing by 50%.
- When looking at the graph you can observe that PEPCK was left unaffected.

Results



Conclusion and Future Directions

- Based on the results of the qPCR and seeing the successful knockdown of dFOXO, PEPCK appears to not be affected by the knockdown.
- Since dFOXO is responsible for the expression of processes like cAMP which regulate enzymes like PEPCK it can be assumed that further research conducted to look at other cAMP regulated processed that dFOXO is responsible for.

References

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