Assessing the Correlation Between DNA Methylation Levels of the ednRB gene using Bisulfite Sequencing and Morphological Color Change of Astatotilapia burtoni

Aliza Mandelbaum, Matt Hackett, Dr. Sebastian Alvarado
Department of Biology, Queens College, CUNY

Abstract
Epigenetics is the study of how the surrounding environment affects gene function. The changes caused by epigenetic mechanisms are reversible and they affect gene expression, but not the organism’s DNA sequence. DNA methylation is a process that regulates gene expression by the covalent transfer of a methyl group to the C-5 position on the cytosine ring of DNA. When portions of DNA are methylated, transcription is silenced and expression of that portion of the gene is reduced. Astatotilapia burtoni, a model species of the African cichlid fish, displays wide variations in morphology and behavior, despite having little to no intraspecies genetic differences. Specifically, there are two typical color morphs: blue and yellow, that correspond to the color morphology of the fish. Change also occurs naturally in the species home environment that this color change is associated with different levels of DNA methylation. DNA can be induced in a laboratory setting, but they are also found in the species. Specifically, there are two typical color morphs: blue and yellow, that correspond to the color morphology of the fish.

Materials and Methods
- 231 fish samples from the species Astatotilapia burtoni were caught and sacrificed on the shores of Lake Tanganyika in Africa. The caudal fin of each fish was removed, labeled, and suspended in ethanol until they were brought back to the lab. They were then washed and placed in individual 1.5 Eppendorf tubes and stored at -80°C until the following step.
- The caudal fin was crushed, and the DNA was extracted. It was then stored in 1.5 Eppendorf tubes at -80°C until the following step.
- The extracted DNA was deaminated using a sodium bisulfite reaction mixture. The converted DNA was then stored in 1.5 Eppendorf tubes at -80°C until the following step.
- PCR amplification of the converted DNA was performed at 60°C using the forward (for) and reverse (rev) ednRB primers listed in Table 1.
- A gel was run to confirm that the primers bound correctly to the converted DNA. This would be portrayed by bands that show up on the gel at the correct base pair lengths for the fragmented DNA.
- Bisulfite sequencing of the converted DNA will be performed using pyrosequencing technologies. The percentage of methylated to unmethylated DNA can then be quantified, as well as how it corresponds to the morphological color of the fish.

Materials and Methods

<table>
<thead>
<tr>
<th>Sample</th>
<th>Con. (ng/μl)</th>
<th>260/280</th>
<th>260/320</th>
<th>Wt. (μg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>307.0</td>
<td>1.86</td>
<td>2.19</td>
<td>3.9</td>
</tr>
<tr>
<td>2</td>
<td>443.0</td>
<td>1.80</td>
<td>2.37</td>
<td>3.8</td>
</tr>
<tr>
<td>3</td>
<td>664.9</td>
<td>1.81</td>
<td>2.38</td>
<td>3.3</td>
</tr>
<tr>
<td>4</td>
<td>522.7</td>
<td>1.84</td>
<td>2.33</td>
<td>2.7</td>
</tr>
<tr>
<td>5</td>
<td>449.4</td>
<td>1.85</td>
<td>2.27</td>
<td>2.5</td>
</tr>
<tr>
<td>6</td>
<td>350.6</td>
<td>1.84</td>
<td>2.28</td>
<td>2.3</td>
</tr>
</tbody>
</table>

Table 1: Concentration of the DNA extracted from the caudal fins of fish samples 1-6. The concentration is measured on a scale of nanogram (ng) per microliter (μl). The 260/280 and 260/320 values indicate the purity of the sample. A ratio ~ 1.8 for the 260/280 value is considered pure. A ratio ~ 2.0-2.22 for the 260/320 value is considered pure.

Future Directions
- These results can be used to further understand and dissect the function of the ednRB gene.
- Do other species have this conserved locus but also display morphological variation in color? If so, can the variation also be explained by DNA methylation patterns?
- When the DNA is methylated, the promoter is unable to bind and begin the process of transcription. A further study can test the promoter with a reporter assay to confirm that DNA methylation effectively blocks transcription.

Acknowledgements
This work was supported by the Undergraduate Science Research Program at Queens College, CUNY, the Honors in Math and Natural Sciences Program at Queens College CUNY, and Macaulay Honors College at CUNY.

Research was Conducted at Queens College, CUNY in Flushing NY.

References
BioRender.com


Unpublished Data from Sebastian G. Alvarado.