Iron-Sulfur Cluster Assembly Proteins in *Landoltia Punctata*

The Pingry School (17) - 2016 WSSP Project
Raymond Chen | Akash Kumar | David Maxwell

Abstract

Landoltia Punctata is a species of duckweed that has numerous future implications today. Duckweed plays a leading role as a potential biofuel source. It can accumulate 40-70% starch which can easily be converted to sugar for fermentation. Duckweed also has a role in bioremediation, using organisms to naturally clean up polluted sites. Duckweed has the ability to grow in contaminated water and degrades pollutants such as lead, arsenate, and halogenated compounds along with extracting nitrogen and phosphate from water waste. By understanding the proteins and the genome on how duckweed functions, we can obtain a better understanding of how to make use of duckweed in order to better take care of our environment and provide natural and efficient fuel

For 2016's WSSP, we worked on *Landoltia Punctata* and sequenced its cDNA, complementary to mRNA which is used in gene expression, allowing us to obtain sequences of proteins instead of noncoding regions In our results, we obtained the DNA and amino acid sequence of an Iron-Sulfur Cluster Assembly Protein.

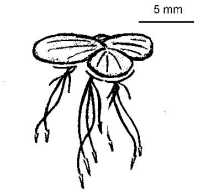

Fig. 1 | Diagram of Duckweed

Methodology

We picked bacterial colonies and grew cultures with overnight incubation. We then isolated the DNA and through processes such as PCR (polymerase chain reaction) and restriction digest. These experiments helped us make an estimate of the DNA sequence length. After isolating the DNA, we sent it in to be sequenced and began the next phase of the process. With this sequence, we analyzed the regions of nucleotides that coded for proteins using the BLAST search programs. We discovered that the protein our sequence coded for was the iron-sulfur cluster assembly protein.

FeSC Protein

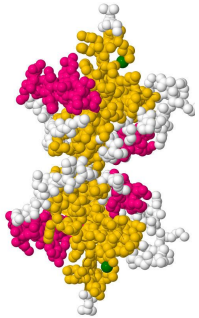

Fig. 2 | Spacefill Model

Fig. 3 | Cartoon Model

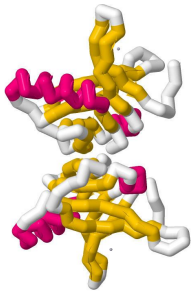

Fig. 4 | Backbone Model

The iron-sulfur cluster assembly protein is a scaffold protein involved in the synthesis of iron-sulfur clusters within chloroplasts. This process is necessary for the growth of cytoplasmic iron-sulfur clusters. Our protein is involved in the biosynthesis of the iron-sulfur cluster. The overall mechanism involves cysteine disulphurase mediated assembly of clusters on scaffold proteins that are then transferred to apo proteins (protein that has not yet formed complex with prosthetic group). A cluster is first formed on a scaffolding protein where frataxin serves as the iron donor and cysteine disulphurase serves as the sulphur donor. The electron transport chain transfers the cluster to a glutaredoxin using ferrodoxin reductase and ferrodoxin which receives electrons from NADH. This is followed by the formation of mitochondrial iron-sulfur proteins and the synthesis of iron-sulfur clusters. They are then inserted into the recipient apoproteins, proteins that lack a prosthetic group, which in this case is the iron-sulfur cluster.

Interactions

By comparing the amino acid sequences of our sequence and the model protein's sequence, the differences and similarities could be seen. In Fig. 5, the dark blue sections show the fully conserved regions while the lightblue sections show areas of similar amino acids. Ligands of the protein can also be seen at the top and the bottom of the model in green. The ligands are mercury atoms.

Using *Arabidopsis thaliana*, a species of Thale Cress, as a model system, we could look at protein interactions using the program Cytoscape. We found that our protein does exists in Arabidopsis, and for good reason, and that It interacts with two other proteins, a glutaredoxin and exocyst complex component.

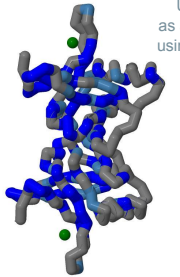

Fig. 5 | Conserved Regions

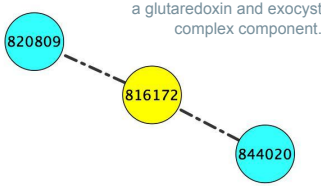

Fig. 6 | Interactions, yellow is FeS protein, right is exocyst protein, left is glutaredoxin.

Citations

1. Crystal Structure of the Ancient, Fe−S Scaffold IscA Reveals a Novel Protein Fold
Patrick W. Bilder,‡, Huangen Ding, and, and Marcia E. Newcomer*Biochemistry 2004 43 (1), 133-139
DOI: 10.1021/bi035440s
2. Iron Binding Activity of Human Iron-Sulfur Cluster Assembly Protein hIscA-1
Lu, Jianxin et al. *The Biochemical journal* 428.1 (2010): 125–131. *PMC*. Web. 21 July 2016.