Investigating location of Histone H3 in Arabidopsis thaliana cells

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Introduction

Histone H3 is a nucleosomal protein involved in chromatin formation and gene regulation. Histone proteins are typically located in the nucleus of the cell, however some studies have shown that histones can bind to the mitochondria and induce cytochrome c release, a protein involved in programmed cell death. This is also backed by predictive software that shows histone proteins being targeted to the mitochondria (Figure 1).

The objective of this study will be to successfully tag histone H3 with a fluorescent protein to determine if it binds to the mitochondria of *A. thaliana* cells. The outcome of this will provide more insight on the function, purpose, and structure of histone within the cells of *A. thaliana*. **We hypothesize that the histone proteins will bind to the outer membrane of the mitochondria.**

Discussion

Research on histone's binding affinity with the mitochondria remains inconclusive. While some groups found that the histone protein binds to the mitochondria, others have found no binding activity at all. These studies use immunoblotting, a method that requires the cells to be ground up and broken down. The grinding process can lead to random binding with free-floating, compatible proteins. Our study seeks to view this binding activity under a fluorescent microscope in order to keep the cell intact.

The outcome of these experiments will provide more insight on the role of these histone proteins within plants. If its binding affinity with the mitochondria leads to the release of cytochrome c, then we can infer its possible roles in programmed cell death within plant cells. Cells that are dead at maturity provide structural integrity in plants, so this research can also lead to a deeper insight on the development of these cells.

Methodology

Transgenic plants with fluorescently tagged histone proteins will be crossbred with transgenic plants containing fluorescently tagged mitochondria. After crossbreeding the plants, their offspring should contain both histones and mitochondria that are fluorescently tagged. We'll observe the tissue of these plants under a microscope and analyze their fluorescence.

Transgenic seeds will be obtained from the Arabidopsis Biological Resource Center. The seeds will be grown in soil under a 16h light/8h dark cycle at room temperature. Experimental results will be viewed under an Olympus CKX41 reflected fluorescence microscope.

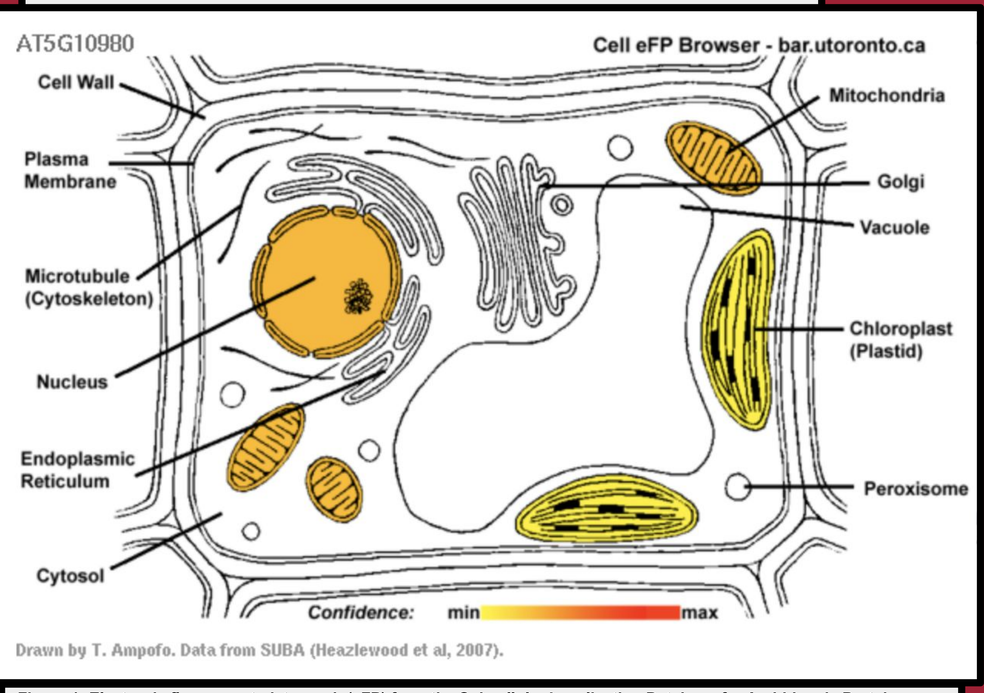

Figure 1: Electronic fluorescent pictograph (eFP) from the Subcellular Localisation Database for Arabidopsis Proteins (SUBA), an online application that predicts the locations of proteins. Predictions are based on AA sequence comparisons.

Expected Results

We hypothesize that the histone protein will bind to the outer membrane of the mitochondria, based on previous experiments that found that core histones bound to and destabilized the outer membrane of the mitochondria. Our expected results would include fluorescent microscopy images of the cells with two different colors of fluorescence, one representing the histone proteins and one representing the mitochondria. We expect to see the two fluorescent colors appear in close proximity to one another.

Figure 2: Photo of adult *Arabidopsis thaliana* plants, also known as the thale cress.

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