The Effectiveness of Cell Fractionation by Differential Centrifugation and Cytochemical Tests

Aaron M Jacobs, Alexandra Digenakis, Andrew Watts, Anthony Shorey, Katarzy Pogorzelska, Maggie Fessler, Nicole Wingard, Daniel S Matasic

Abstract: All plants contain plastids, which are organelles that can play a role in anything from starch storage to the process of photosynthesis. The types of plastids present depend on the type of cell; this experiment focuses on pea cells and spinach leaf cells, and more importantly the location of that cell. Plastids are known to interconvert between one another depending on the environment of the cell. This experiment focuses on how to separate and distinguish between these types of plastids from other organelles in the cell using cell fractionation by differential centrifugation and cytochemical tests in order to determine in future experiments if interconversion occurred between plastids. Here we show that these tests are effective tools for distinguishing between cellular organelles. Based on these findings, this research is important to the field because it shows an effective way for all scientists to distinguish between cellular organelles in a controlled manner.

Introduction.

Plants, along with many other types of organisms, have different types of plastids in a cell along with the better-known structures of a cell like the nucleus and mitochondria. Plastids came into existence through various processes of endosymbiosis, which was when early prokaryotes engulfed bacterium and instead of metabolizing them used them for actions like photosynthesis. Two crucial plastids found in plant cells include amyloplasts and chloroplasts. An amyloplast is a starch storing plastid, while a chloroplast is a photosynthetic plastid that contains thylakoid membranes. Depending on the type of cell, or more importantly the location
of that cell, the plastid may vary\(^1\). Plastids are known to interconvert between one another depending on the environment of the cell. When Cells with a majority of chloroplasts were moved from a light rich environment into a dark environment, those chloroplasts interconverted into amyloplasts by losing their thylakoids and gaining starch granules\(^2\). The difficulty is being able to distinguish between the chloroplasts and amyloplasts and how to even find out if they did differentiate.

Cellular fractionation by differential centrifugation is a process that separates the organelles of interest so they can be further studied. At different speeds and amounts of time left in a centrifuge, certain organelles will separate in the form of a pellet leaving the rest of the organelles in the supernatant. Depending on the size, shape and density of the organelle it will separate at lower or higher speeds. The nuclei for example has a density of 1.32g cm\(^3\) and is about 10um in size, this would lead one to predict that a nuclei would separate at lower speeds compared to organelles like chloroplasts since they only have a density of 1.32g cm\(^3\) and are 2x5um in size. In order to determine the actual organelles that are present requires a different technique, which is a cytochemical test. Chemicals such as methylene blue and Lugol’s iodine are used during cytochemical testing to help distinguish between the various organelles being observed under the microscope. Methylene is used to stain DNA, revealing organelles like mitochondria and nuclei. Lugol’s iodine is used to stain starch, which is meant to stain amyloplasts for better visualization\(^3\).

This experiment focuses on the implications and effectiveness of differential centrifugation and cytochemical tests. Assuming everything is run correctly, there are a few outcomes that are expected from this experiment. The pea cells should contain more amyloplasts since peas have a starch content between 53.61-57.23\(^\%\)\(^4\). Spinach leaves on the other hand are
expected to have a higher content of chloroplasts, since plant leaves are used for photosynthesis, which is a process that requires chloroplasts\(^5\). Due to the size and density of nuclei and amyloplasts, the centrifuged contents of pellet one and two should contain higher quantities of these organelles, compared to the third pellet, which should contain mostly chloroplasts\(^3\). This work is to determine if standard cell fractionation by differential centrifugation and cytochemical tests are effective ways of isolating cellular organelles for future studies, including the interconversion between chloroplasts and amyloplasts.

**Methods.**

The sample had to first be prepared, the lab TA Daniel Matasic prepared the cell homogenate, specifically my group had pea cell homogenate. A cell homogenate is prepared by blending up the peas and collecting from the blended material the supernatant with the desired cellular materials to be further tested. Cellular fractionation by differential centrifugation was then used to separate the cellular organelles. To separate pellet one, the homogenate was spun centrifuged at a RCF of 100g. The supernatant was collected and the supernatant was then centrifuged at a RCF of 800g to separate out pellet two. The supernatant was then collected and centrifuged at a RCF of 10,000g to separate the third pellet from the supernatant, all of which were spun for 10 minutes each. Once separated the three pellets and one supernatant had to be set on slides\(^3\).

Cytochemical techniques were then used to distinguish between the slides. One slide from each pellet and the supernatant either received methylene blue, Lugol’s iodine or no stain, making three different slides for each constituent. Each stain reacted when certain components were present; methylene blue found DNA and Lugol’s iodine attached to starch. Then by using microscopy we viewed, what we thought were accurate depictions of the whole slide and drew
out what we saw. Since my group used 10x magnification for the pellet one and 40x for the rest, we had to multiply the amount found in the 40x pictures by 4 to make them all equal. An abundance scheme was created to determine the amount of observed organelles in each slide picture; stars were used as the measuring system. No stars meant no organelles, one star meant a little, two stars equaled some and three stars equaled a lot. After examining each slide the star denotations were given to what was observed on the slide.

**Results.**

There are some major patterns that arose between the various data sets and figures. Table 1 and Figure 1 show the abundance of organelles in pea cells, the methylene blue slide decreases with every centrifugation, representing a decrease in nuclei. The important comparison comes between the Lugol’s iodine and the unstained. The Lugol’s iodine line from figure 1 shows a higher quantity of organelles, which is representing a larger amount of amyloplasts. When this is compared to the unstained, the abundance is for the most part less then that of the Lugol’s iodine.

Table 2 and Figure 2 show some patterns seen from spinach leaf cells, first the unstained doesn’t show any change. This could mean that the amount of organelles in the unstained, which represents chloroplasts, is very low and isn’t changing with centrifugations. The methylene blue shows some pattern, when centrifugations take place the amount of organelles decrease, representing a decrease in the amount of nuclei due to the amount of centrifugations. The Lugol’s iodine from Table 2 and Figure 2 doesn’t show any pattern, the amount of organelles both decreases and increases as a result of the centrifugations being done.

When comparing both Figure 1 and Figure 2, some patterns can be found. For the most part the amount of organelles observed from the Lugol’s iodine slides from the pea, meaning more amyloplasts, is higher then the amount found in the spinach leaf cells. The amount of
chloroplasts, unstained organelles, doesn’t really show a relationship between the Figure1 and Figure 2. As for the amount of nuclei, methylene blue organelles, both show that by the end of centrifugations there is a very small amount of methylene blue stained organelles.

**Discussion.**

This research shows that cellular fractionation by differential centrifugation is an effective way to separate the organelles within a cell and cytochemical tests are effective ways of identifying organelles more easily. There is some evidence that supports differential centrifugation, including the fact that in the pea cells, the amount of nuclei, methylene blue, was larger in pellet one when compared to it’s more centrifuged constituents, which follows what was expected, the denser nuclei pelleted first. Also, the expected outcome was that the pea cells would have more amyloplasts, which the data suggests, except for the jump of Lugol’s iodine in Figure 2 supernatant 3. Figure 2’s data consistently decreases for amyloplasts up till that point, which could suggest an error of some sort. The amount of chloroplasts in the spinach leaves was expected to be higher then that of the pea cells, the contrary was observed. Figure 2 shows a straight horizontal line for unstained, chloroplasts, at one star showing no conclusive data and leads us to believe that another error occurred. The cytochemical staining did bind effectively to the organelles and through microscopy the organelles could be easily seen. Some evidence that confirms this is the fact that the methylene blue showed a consistent decrease in both cell types from the first pellet to the later pellets, except in Figure 2, pellet 2. This decrease suggests that the methylene blue represented a larger and denser molecule and since the nuclei is the largest and one of the denser organelles\(^3\), it is expected that it would sink first. Lugol’s iodine shows a smaller decrease from the first centrifugation in Figure 1 compared to the nuclei, this would suggest that the Lugol’s iodine stains the amyloplasts since they are a smaller then the nuclei\(^3\).
The chloroplast data doesn’t show much of any relationship in Figure 2 and figure one doesn’t really show anything conclusive. All these questionable data points could be due to error or some other outside influence.

As mentioned above, some errors seemed to have occurred. The first source of error that could have affected the results could have been that the portion of the slide that was being observed under the microscope wasn’t an accurate representation of the population of organelles on the whole slide. This could be an error because if the portion of the slide that was selected shows a lot or not enough of the population this could provide the wrong analysis of the quantity of organelles on that slide, skewing the data. Centrifugation errors could have also occurred, if the centrifuge wasn’t balanced correctly the pellet that was formed may not contain all of the organelles that it should. Also, during centrifugation organelles might have trapped other organelles underneath them as a result of them sinking to the bottom of the tube. Our star abundance scheme also wasn’t the most accurate since there isn’t really a numeric range behind the stars, they are very subjective to the person designating them. Our group used 10x magnification for the first pellet and then 40X for the rest, this meant that we had to calculate the amount of organelles found in the 40x magnification by 4 which could have really skewed our data. A lot of the error in this work was a result of human error, which is expected but should be minimized. Future improvements could include a forming a more precise abundance scheme and also possibly changing the centrifugation speeds to possibly get a better result as far as the organelles being pelleted goes.

From here, trials would be run, which would change the conditions so the plastids would interconvert, the procedures described in this work could be carried out to really see if interconversion occurred. By knowing if interconversion is possible, scientists could create
possible super crops that have high amounts of chloroplasts, making a plant grow faster, and the high amounts of amyloplasts, which would create larger crop yields.

References.


Tables and Figures.

Table 1.

<table>
<thead>
<tr>
<th>Plant Cell Type: Pea</th>
<th>Abundance of Organelles Observed</th>
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<tbody>
<tr>
<td></td>
<td>Cell Fraction</td>
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<tr>
<td></td>
<td>Nuclei (Methylene Blue)</td>
</tr>
<tr>
<td>Pellet 1</td>
<td>★★★</td>
</tr>
<tr>
<td>Pellet 2</td>
<td>★★</td>
</tr>
<tr>
<td>Pellet 3</td>
<td>★</td>
</tr>
<tr>
<td>Supernatant 3</td>
<td>★</td>
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Table 1: Abundance of Organelles in Pea Plant Cells. This data refers to the abundance of organelles found in pea plant cells as a result of differential centrifugation. Cytochemical tests were used to then identify the different organelles that were being compared.

Figure 1.

Figure 1: Scatterplot Representing Data from Table 1. This graph represents in a more visual way the data from Table 1, so comparisons can more easily be made.
Table 2:

<table>
<thead>
<tr>
<th>Plant Cell Type: Spinach</th>
<th>Abundance of Organelles Observed</th>
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<tbody>
<tr>
<td>Cell Fraction</td>
<td>Nuclei (Methylene Blue)</td>
</tr>
<tr>
<td>Pellet 1</td>
<td>★★</td>
</tr>
<tr>
<td>Pellet 2</td>
<td>★★★</td>
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<tr>
<td>Pellet 3</td>
<td>★</td>
</tr>
<tr>
<td>Supernatant 3</td>
<td>★</td>
</tr>
</tbody>
</table>

Table 2: Abundance of Organelles in Spinach Leaf Cells. This data represents the amount of organelles located in the spinach leaf cells and how, due to the amount of centrifugations done, the number of organelles vary. Cytochemical stains were used to help distinguish between the various organelles.

Figure 2:

Figure 2: Scatterplot Representing Data from Table 2. This scatterplot allows for a better visual representation of the patterns that can be seen from the Table 2 data.