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## Cell Death in Algae

Apoptosis is the process of necessary and programmed cell death that is used to eliminate damaged or unwanted cells in the body. Apoptosis occurs in all multicellular organisms; without this process cells can grow at an uncontrolled rate often leading to tumor growth and sometimes cancer. (Austin) The main indicator in both plant and animal cells starts with the nucleus. Chromatin condenses, DNA fragments, and membranes bleb, these steps indicate the stages of apoptosis. (abcam) While this organelle and its process in apoptosis is similar between animal and plant cells other organelle functions are different among these different cells. While the mitochondria is the primary organelle used in apoptosis in animal cells and plant cells, the process becomes much different when considering the chloroplasts. The chloroplasts are not a main component in the process of apoptosis and could possibly work along with the mitochondria. When light is introduced the chloroplasts support the mitochondria in this process by producing reactive oxygen species. (Aken and Breusegem, 2015) In certain plants such as many algae species and hornworts, there is another compartment found within the chloroplast known as the pyrenoid. The pyrenoid is a compartment located within the chloroplast that is known to maintain carbon dioxide levels within. Not much is currently known of pyrenoid function during apoptosis although it is believed that the pyrenoid could contain an enzyme that triggers the initial steps of apoptosis. (Bidle, 2016)

The four types of algae being evaluated are *Pandorina*, *Volvox*, *Chlorella*, and *Rhodochorton*. *Pandorina* is a genus of green algae that is found in freshwater environments. Similar to other algae they are multicellular, and can reproduce asexually or sexually. Depending on the species of *Pandorina*, they often contain a large cup-shaped chloroplast that either contains

a single basal or multiple pyrenoids.(Guiry, 2020) Similar to the Pandorina, the Volvox is also a genus of green algae.They dwell in freshwater, often found in puddles, ditches, ponds, and other fresh, shallow bodies of water. Volvox are multicellular and are found to reproduce sexually or asexually. Each spherically shaped cell contains a pair of vacuoles along the base as well as a large chloroplast with a single pyrenoid. (Guiry, 2020) Chlorella is another genus of green algae. Unlike the previously mentioned, Chlorella is unicellular and produces asexually and rapidly. They do still contain a pyrenoid. Chlorella are mostly freshwater dwelling organisms. (Guiry, 2015) The last algae, Rhodochorton, is a genus of red algae. This multicellular organism contains many chloroplasts but does not contain a pyrenoid. Rhondochorton is typically found in marine environments often growing on rocks in intertidal zones, and off other algae, although it can be found dwelling in freshwater environments. (Wikipedia, 2020)

When analyzing the evolution of eukaryotes, it has been proven difficult to determine the exact order that the evolution of these organisms occurred due to lack of fossilized organelles. It has been interpreted by the scientific community that this is due to the organelles decaying faster than the rate of fossilization however, there is very little evidence supporting this. Due to the scarcity of fossilized organelles, it was also deemed impossible to prove this statement true. Taking information into account, a group of scientists conducted an taphonomy experiment using the Pandorina, Volvox, Chlorella, and Rhodochorton algae groups to test whether organelles decay too rapidly for fossilization.

To begin the experiment the algae were euthanized using  $\beta$ -mercaptoethanol (BME) to prevent the algae's enzymes from self-digesting their cells. Then placing the algae in an environment that mimicked their natural ones, they decayed for six weeks. These experiments were also done under oxic and anoxic conditions to serve as a control for aerobic activity. There

was no difference in the decay patterns between the groups, although the anoxic conditions showed slower results than in previously studied experiments. (Carlisle, Jobbins, Pankhania, Cunningham, Donoghue, 2021) Over the course of the experiment, the scientists examined the changes in the different algae's organelles. These changes included nucleus visibility, pyrenoid visibility, chloroplast holes and/or thinning, and whether the chloroplast or cell itself collapsed.

Volvox algae (*V. aureus*) dwell in colonies that number anywhere from five-hundred to ten-thousand cells. (Carlisle, Jobbins, Pankhania, Cunningham, Donoghue, 2021) After death the cells themselves maintained their shape, but gradually became more spaced out after several weeks, forcing some colonies to rupture or become misshapen. The chloroplast and nuclei remained for a longer period of time, usually after at least six weeks. The nuclei were seen disappearing before the chloroplasts but stayed more consistent in appearance. The chloroplasts however, thinned before fragmentation and eventually disappeared. The pyrenoids of the Volvox were the least resistant to decay.

Pandorina algae (*P. morum*) although structurally similar to Volvox algae, contain much smaller colonies numbering around four to thirty-two cells per colony. (Carlisle, Jobbins, Pankhania, Cunningham, Donoghue, 2021) The colonies were seen collapsing almost immediately, with the decreased volume and separation of the cells. The nuclei still remained visible six weeks after death, but their abundance gradually decreased. The chloroplasts were the most decay resistant out of all organelles but showed evidence of irregular shape, holes, and thinning. Pyrenoids ended up being the least decay resistant and were usually fully decayed by the third week after death.

Chlorella algae has a similar structure to those of the individual Pandorina and Volvox cells. The cells of the Chlorella algae showed little to no change. The nuclei and pyrenoids remained during the six weeks of evaluation and showed no evidence of degradation. The chloroplasts were seen escaping the cell if they ruptured but otherwise they would collapse leading to holes, thinning, and ultimately disintegrate.

The Rhodochorton red algae remains the most diverse from the previous algae. Rhodochorton do not contain pyrenoids, so they were not observed during the study of this algae. The chloroplast often blocks the view of the nuclei in the cells however, nuclei were still observed after six weeks of study. The chloroplasts collapsed quickly forming irregular shapes and holes, often resulting in a disc-like shape that remained in the cell for the six weeks it was studied after death.

Overall the experiment concluded that nuclei were the most persistent throughout the entire experiment. While the chloroplasts often became deformed they remained the most resistant against decay. The pyrenoids however, would likely not be visible in fossils unless it was present in the surrounding chloroplasts. This study indicates that the organelles present in previous landmark Eukaryotic fossils could very well be nuclei or chloroplasts. This is something that was previously rejected by scientists who have studied these fossils. These discoveries are extraordinary and can serve as a stepping stone to further determining past Eukaryotic development.

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