Inorganic Phosphate (Pi) is a vital molecule in cellular metabolism, that plays a crucial role in various biochemical processes. It comprises one phosphorus atom bonded to four oxygen atoms in a tetrahedral arrangement**.**[1] They are an “essential nutrient to living organisms.” [2] Furthermore, the two main roles of inorganic phosphate in cellular metabolism are its involvement in ATP (adenosine triphosphate) synthesis and hydrolysis. [3]. As known, ATP is cells' primary energy currency, which provides energy to various cellular processes. Inorganic phosphate is also utilized during the phosphorylation of ADP (adenosine diphosphate) which forms ATP through processes like oxidative phosphorylation and substrate-level phosphorylation. In addition, the inorganic phosphate's role in cellular signaling and regulation is crucial as it acts as a phosphate donor that regulates the reactions of enzyme activity, protein function, and gene expression.[4]. While its significance spans across life forms, studies on Pi metabolism have predominantly focused on bacteria, yeast, and plants. In bacteria, Pi is stored in polyphosphate granules, whereas yeast and plant cells primarily store Pi in vacuoles. However, our understanding of intracellular Pi metabolism and signaling in animals remains limited, despite significant knowledge about the hormonal regulation of circulating Pi.[5]

The features of PXo bodies are investigated using fluorescent imaging techniques. This involves assessing their size and morphology, typically by tagging the PXo phosphate transporter with fluorescent markers. One method involves attaching a green fluorescent molecule to the protein's N-terminus and an HA-tagged red fluorescent molecule to its C-terminus. When these two fluorescent signals overlap, new colors like yellow and blue emerge, aiding in visualization. Notably, a blue nuclear stain (DABI) is used to highlight cell nuclei.[6] For characterization, electron microscopy with immunogold labeling is employed to pinpoint PXo protein locations within these organelles. The presence of yellow fluorescence indicates acidic conditions, suggesting an association with LysoTracker Red (LysoT) and lysosomal activity.[7] Conversely, the absence of red-green overlap with lamp 1, a lysosome marker, signifies a distinct identity from lysosomes. Nile Red staining reveals lipid content, with overlapping red-green fluorescence indicating lipid presence.[8] PXo's overlap with Man II, a Golgi apparatus marker, aids in distinguishing Golgi-associated organelles. The endoplasmic reticulum (ER) and Golgi play vital roles in cellular pathways, especially phospholipid synthesis due to their membranous structure.[9] Glycosylation, a process occurring in the rough ER, is indicated by yellow staining. Phospholipid tracer (P-Cho) staining confirms phospholipid composition within PXo bodies. Furthermore, the absence of yellow staining with dextran, an endocytosis marker, suggests that PXo bodies are not involved in endocytic processes.[10] In summary, PXo body characterization includes assessing acidity, lysosomal features, lipid content, Golgi association, glycosylation, phospholipid composition, and endocytosis pathway involvement.

Moving on, for the PXo to be able to regulate the levels of inorganic phosphate in the cytoplasm, it all begins with Fluorescence Resonance Energy Transfer (F.R.E.T).[12] In a normal F.R.E.T., there are two fluorescence molecules. The first color, blue, is the excited. Then it passes on to the color yellow. If the process is successful, you will see a yellow color. But if the energy transfer is unsuccessful, a blue color will appear instead. This indicates that the blue color is still excited to receive that light but its energy did not successfully go over through the yellow color.[13] A sensor for intracellular inorganic phosphate is called FLIPPi. So, in the FLIPPi model that has been created, a binding protein is used for the inorganic phosphate.[14] Where the two fluorescence molecules are stuck in there with enough space for a phosphate. Furthermore, once the phosphate is at high levels in the intracellular environment, extra phosphate groups bind to a green-binding protein, which blocks the energy-resonant transfer between the blue and yellow fluorescent molecules. In the end, only a pure blue signal is shown.[15]

Unfortunately, the panels of cells and what they did are not directly shown under the microscope, but the heat maps of every panel provide enough information to show the difference in coloration. The heat maps help determine if the PXo regulates levels of inorganic phosphate in the cytoplasm, either high or low.[16] In addition, seen on the heat maps is a red area which determines where the fluorescence had its highest peak. If what comes after it is a yellow fluorescence molecule, it means that the inorganic phosphate could not bind in between the molecules to prevent the F.R.E.T.[17] Moreover, the PXo affects the inorganic phosphate in a way that it pumps phosphate inside of the cytoplasm because its F.R.E.T ratio is lower. As evidence shown by the panels, there are more blue fluorescence molecules than yellow fluorescence molecules.[18] Summing up Figure 3, to know that PXo regulates levels of inorganic phosphate in the cytoplasm, only the blue fluorescent molecule is shown if phosphate levels are high in the cytoplasm. But if the phosphate levels are low in the cytoplasm, only the yellow fluorescent molecule is shown.

In connection to the levels of PXo in inorganic phosphate, there are two things to help determine the formation of PXo bodies based on the availability of inorganic phosphate: number and size.[19] If the PXo bodies are with the inhibitor PFA, phosphonoformic acid is an inhibitor of the cellular inorganic phosphate uptake. The size of it appears to be smaller than when it does not bond with any inhibitor PFA. As for the number, green fluorescent protein is attached to the PXo protein with the use of the dappy nuclear stain. In normal cells, the PXo body cells are present as a green fluorescent molecule. In the presence of the inhibitor phosphonoformic acid (PFA)[20], which inhibits cellular phosphate uptake, PXo bodies appear smaller compared to their uninhibited counterparts. Moreover, the number of PXo bodies can be tracked by attaching green fluorescent protein to the PXo protein using the dappy nuclear stain. If the phosphate levels drop in the cytoplasm of the different types of PXo bodies found, only the yellow fluorescent molecule is shown. As mentioned in Figure 3[21], if the levels of the phosphate are high in the cytoplasm, only the blue fluorescent molecule is shown. A change in the PXo bodies occurs when the red fluorescence molecule is higher than the blue fluorescence molecule.

Ultimately, the PXo bodies, do not form distinct organelles with a unique biochemical function in the cell. Instead, they are considered to be specialized structures within peroxisomes. Peroxisomes themselves are membrane-bound organelles that play crucial roles in various metabolic processes, including lipid metabolism, detoxification of harmful substances, and regulation of reactive oxygen species (ROS). PXo bodies are thought to be clusters of peroxisomal membrane proteins involved in antioxidant defense, particularly in protecting against oxidative stress. However, PXo bodies are not recognized as separate organelles because they lack a distinct membrane. Instead, they represent a functional compartment within the peroxisome where specific biochemical reactions related to antioxidant defense occur. Therefore, while PXo bodies have a unique biochemical function related to antioxidant activity, they do not qualify as distinct organelles within the cell due to their structural integration within peroxisomes.

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