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Analysis of psToc 34 of the Translocon at the Outer Envelope Membrane of Chloroplasts

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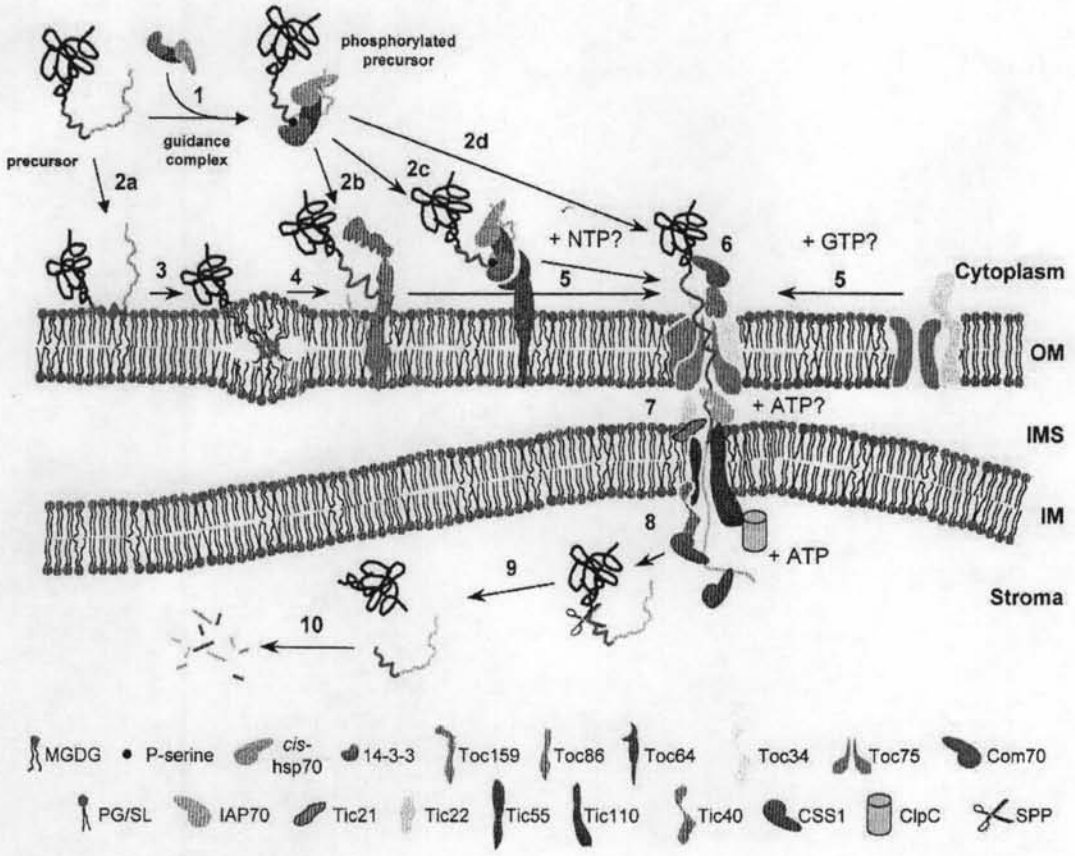

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Approved: _____

Before the evolution of multi-cellular organisms and long before the first animals swam, crawled, or walked on the surface of the Earth, single celled prokaryotic organisms called cyanobacteria were one of the predominant living organisms. These bacteria were the first to discover a way to oxidize water by their photosynthetic machinery, which eventually gave rise to our current oxygen-containing atmosphere. According to the theory of endosymbiosis, larger, eukaryotic cells engulfed the cyanobacteria, possibly at first as a source of food, yet over time the cyanobacteria were maintained and probably providing renewable energy via their preexisting photosynthetic properties. In land plants, the once free-living cyanobacteria have become a family of organelles known as plastids, with the chloroplast being the organelle now providing energy through photosynthesis.

Over time the acquired cyanobacteria lost or gave-up certain biological capabilities that were no longer needed. One of the lost abilities was the genetic ability to express proteins from its original cyanobacterial genome. Through some selective process, the plastid lost most of its DNA and appears to have transferred much of it to the host nucleus. These nuclear-encoded proteins that were originally found in the plastid/cyanobacterial genome must now be efficiently and selectively transferred from the cell's cytoplasm back into the chloroplast. Investigating how these different proteins are synthesized outside in the host cytoplasm, then find their way to the chloroplast surface, and then cross the organelles membrane via some selective process is the subject of this summer internship.

The "information" that directs the organelle protein to specific sites on the envelope of the plastid (the outer membrane of the organelle) appears to be found within an extra part of the protein which has been added to the "front end" (N-terminus) of the protein. This short sequence is called a transit peptide. When the original protein and the transit peptide are attached together,

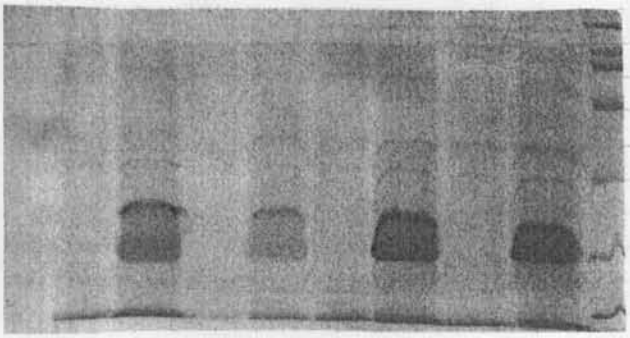
the complex is termed a precursor protein. This protein complex is slightly larger than the original protein that was once encoded by the cyanobacteria. The transit peptide functions like a specific "zip code" that ensures the correct delivery of a package to a specific house. The cell must contain some type of "delivery system" akin to the postal service to help sort and deliver the appropriate mail to the correct address. Somehow the transit peptide is "read" by this "delivery system" to ensure that the precursor arrives at the chloroplast membrane. Once the protein is delivered, then there are "gatekeepers" that direct precursor protein through a special membrane opening known as a translocation apparatus. Scientists now know that the gatekeepers that regulate entrance to the chloroplast are a special class of proteins, known as receptor GTPases.

This translocation apparatus was the focus of my internship. The apparatus contains about twenty proteins in all; however I focused mainly on psToc34. Current research cannot specify if psToc34 is the first protein of the apparatus to interact during protein import. Previous studies have stated that Toc159 is the first receptor while other studies state that the transit peptide, attached to the organelle protein, interacts with lipids away from the translocon and later incorporates somehow into the translocon.

The first step in studying psToc34 is the manufacturing and purification of the protein. First DNA is transformed into competent cells of E. Coli. The DNA contains both a resistance gene to the antibiotic ampicillin and the gene for psToc34. The cells containing the DNA are spread onto agar plates containing Luria broth media and ampicillin. Since the plate media contains the antibiotic ampicillin, the only colonies that will grow are ones that contain the ampicillin resistance gene and the psToc34 gene. After selecting five colonies from the Petri dish, a colony

screen is performed to determine which of the four colonies makes the most psToc34 protein when induced with IPTG.

The image below is a colony screen for a mutant of psToc34. For each colony a uninduced and induced sample are run in successive lanes. The effect of the induction of IPTG can clearly be seen by the absence or presence of thick dark lines. The thick dark lines are the induced samples from each colony. These lines represent the amount of psToc34 that will be made by the bacteria when induced. The colony that has the thickest and darkest line is the colony that will produce the most psToc34 when induced. The goal is to find the colony that will not only produce a large amount of the desired protein, but also not produce large amounts of house proteins of E. Coli. For this colony screen the third colony is the best choice for continued growth and subsequent purification.



(from left to right: lane 1 is SSB, lane 2 is colony 1 uninduced, lane 3 is colony 1 induced, lane 4 is colony 2 uninduced, lane 5 is colony 2 induced, lane 6 is colony 3 uninduced, lane 7 is colony 3 induced, lane 8 is colony 4 uninduced, lane 9 is colony 4 induced, lane 10 is a molecular weight marker.)

After finding the most productive colony, six liters of cells are grown from the same strain. When the liter flasks reached a specific optical density of 0.4 nm, they are induced IPTG, and left for four hours shaking in a warm room. After four hours the contents of the liter flasks is spun in a centrifuge in order to isolate the cells and protein from the media. The supernatant is discarded and the pellet is harvested. The final step in recovering the wanted protein is the separation of the desired protein psToc34 in the pellet by its affinity for Cobalt. The affinity is artificially made by adding several histidine residues to the tail of the protein, thereby creating a His-tag that can be easily snatched out of a mixture of proteins and E.Coli parts by a strong metal cation like Cobalt. After separation and purification of the protein is complete, the sample of psToc34 is sent to a lab in Japan that analyzes the structure of the protein through spectrometry. The results we have received so far state that psToc34 may dimerize in vitro. No other previous studies of psToc34 have shown that the protein dimerizes. If the results do indeed consistently point to this conclusion, the Bruce lab can be accredited with discovering a previously unknown phenomenon. This possible discovery shows that much can be learned about this import protein with continued study.