Elucidating the Mechanism of Nitrate Uptake in Plants

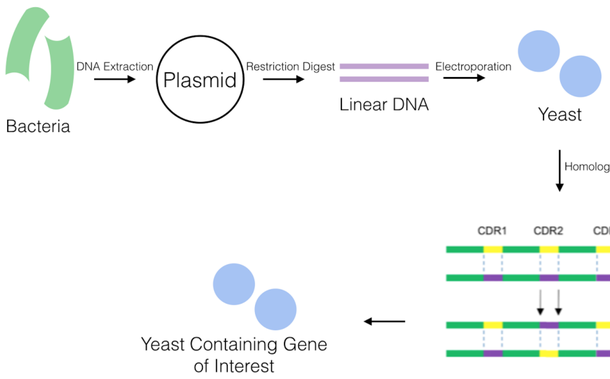
Jasmine Lee, Maria Sofia Ospina, Yajaira Perez-Lascurain, Denisse Garza, Aaron McGrath, Charles Morgan, Dr. Geoffrey Chang

Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California San Diego, 9500 Gilman Drive, La Jolla, California

Abstract

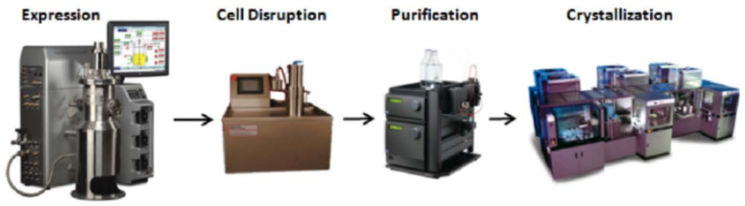
The objective of this study was to examine proteins that act as transporters, specifically CHL1. Cells create protein transporters that experience conformational changes in order to move substrate molecules across the membrane. This activity helps cells obtain the nutrients around them. During the three weeks in Dr. Chang's lab, we focused on purifying CHL1, which controls the uptake of nitrate in plants. CHL1 has the capability of switching between low affinity and high affinity depending on varying nitrate concentrations within plants, which is unlike most other cells that require two transporters for each type of affinity. This process of shifting affinities occurs when CHL1 is phosphorylated and dephosphorylated at Thr 101. Understanding the function of this nitrate transporter provides the opportunity to potentially increase crop yields, reduce the need for nitrogen fertilizer, and lessen marine toxicity by lowering the amount of nitrate that is washed off into the water systems.

Methods– *Pichia pastoris* Transformation



The process of preparing samples for structural determination begins with the transformation of bacteria, which involves adding a circular plasmid that is later converted into linear DNA. At this state, the linear DNA is electroporated into *Pichia pastoris* yeast, which undergoes homologous recombination. The outcome is a yeast strain containing the gene of interest, which is placed into a fermenter.

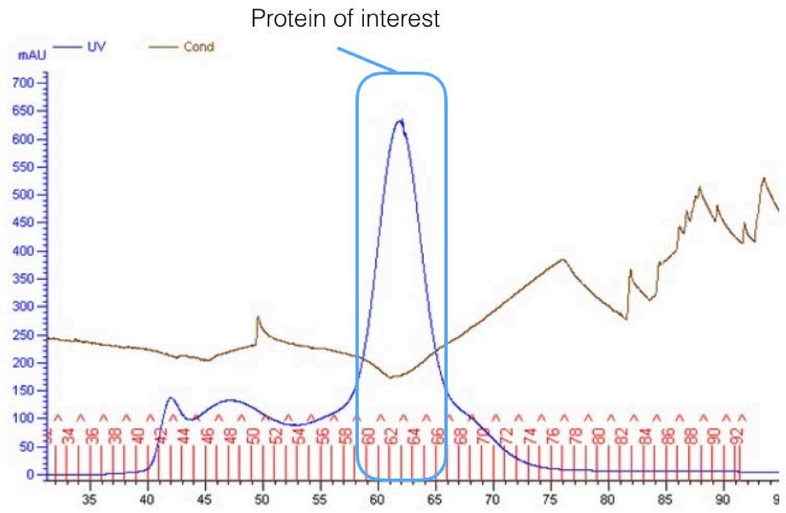
Methods– Protein Expression and Purification



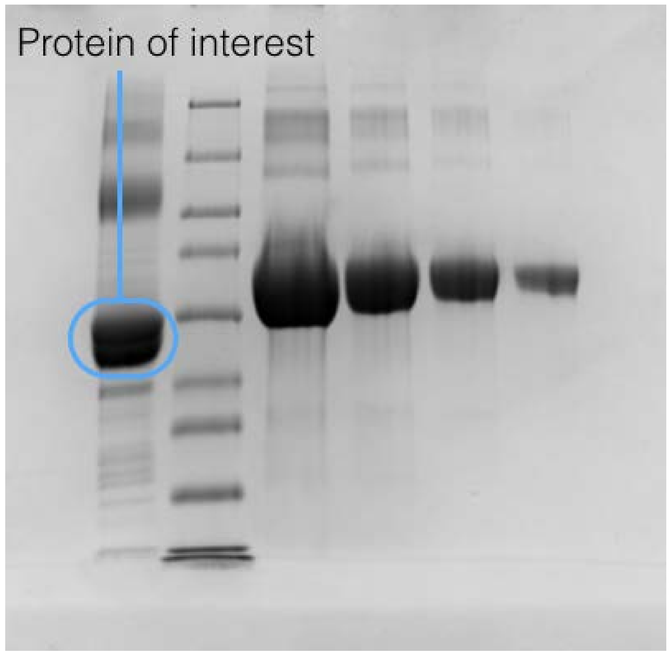
When the cells have grown to a certain point, they are induced and harvested from the fermenter. The collected liquid is then spun in centrifuges in order to remove any substances besides the cells. Afterwards, the cells are disrupted to isolate the membranes in which the protein is embedded. Membrane preparation follows, with a slow spin that leaves behind a soup of soluble portions and lighter organelles and a fast spin that results in pellets comprised of membranes. The final step is protein preparation, which includes solubilizing the membranes with detergent to extract the protein and purifying the protein using affinity chromatography.

Results

The process of purifying protein samples requires affinity chromatography, which takes place in an AKTA FPLC instrument. The protein is also passed through a nickel affinity column in order to separate any impurities from the protein sample.

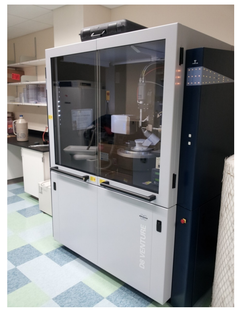


In the chromatogram above, the large peak that is circled illustrates the elution of protein from the column. However, to ensure that the eluted protein is in fact the protein of interest, it is necessary to run a SDS-PAGE gel.



The first lane of the SDS-PAGE gel displays the protein CHL1 T101A, which, as expected, ran at around 52 kD. The third band down is likely to be a dimer. BR Spectra, the ladder/marker, lies in the second lane. To the right of the BR Spectra is the BSA protein in different concentrations: 20 mg/mL, 10 mg/mL, 5 mg/mL, and 2 mg/mL. After comparing the protein to BSA standards, we concluded that the protein was approximately 10 mg/mL.

Future Development



Due to the lack of time, we were unable to carry out the structural determination of the protein CHL1. If we had a longer period of time to complete all the phases of the experiment, we would have proceeded with the crystallization of our protein CHL1 in the machine depicted on the left, a X-ray diffractometer.

The process would first require testing the protein in various environments to discover the optimal conditions for the crystallization of the protein. From there on, the X-ray crystallographer would be used to determine the structure of the protein, which provides the opportunity to understand the protein's function.

Conclusion

CHL1 is a protein transporter that controls the uptake of nitrate in plants and is able to switch affinity, an extremely special feature. This protein transporter is significant because of the positive impact it could have on agriculture. In order to determine the structure of the transporter, bacteria is transformed, and a yeast strain containing the gene of interest is created. Afterwards, the yeast strain is grown in a fermenter, induced at the appropriate time, and harvested. The following steps involve centrifugation, disruption, membrane preparation, and protein preparation using affinity chromatography. Finally, an SDS-PAGE gel is run to make sure that the protein of interest is in the sample.

Overall this was an incredible experience, in which we were able to learn in a hands-on environment rather than in a normal school setting. Additionally, the staff in the lab have been kind and helpful.

References

1. Tsay, Yi-Fang. *How to Switch Affinity*. N.p.: Macmillan, 2014. Nature. PDF file.
2. Kahn, Kalju. "A2-X2-60." *Chem 112L*. UCSB, 2011. Web. 27 July 2016.

Acknowledgments

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