Purifying Emerin for Single Molecule Imaging

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ABSTRACT

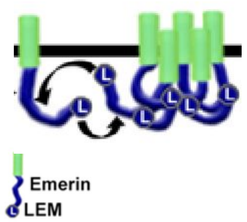
Emerin is a protein which is involved in mechanobiology of the nucleus and is linked to muscular dystrophy diseases. Emerin is a LEM-domain protein that can self-assemble into nanoclusters at the nuclear envelope of human cells in response to mechanical stress. Emerin self-assembly protects the nucleus against excessive mechanical stress, but the molecular mechanisms controlling the assembly/disassembly process have not been clearly defined. To understand how emerin takes part in molecular scaffolding, Emerin to Emerin interactions are currently being studied by the Pinaud Lab. As a part of this umbrella project I optimized procedures for expressing, purifying, and tagging protein Emerin to study Emerin-Emerin interactions on a molecular level with single-molecule imaging.

This process included growing a culture of transformed E. Coli while withholding the induction of the unnatural amino acid, OPGY UGG. The unnatural amino acid is used for site-specific fluorescent labeling, and also plays a role in the production of this modified Emerin. Without the unnatural amino acid the emerin is expected to not be produced, as well as not be labeled. After culturing the protein solution was purified to extract the emerin by running the solution through an IMAC column. This was done since Emerin has a 10x His-tag that has an affinity to nickel. This phenomenon allows for the isolation of emerin in a purified solution. Samples from each step were run through a gel and a western blot and analysed for the presence of Emerin which was not expected. The data indicated the faint presence of emerin in one of the purified fractions. This fraction was then labeled by click chemistry labeling and then imaged beside a positive control. This revealed that the emerin present in the negative control was not labeled due to its lack of the unnatural amino acid. The production of the negative control was largely a success apart minor issues such as the leaky termination causing faint emerin production.

OBJECTIVE

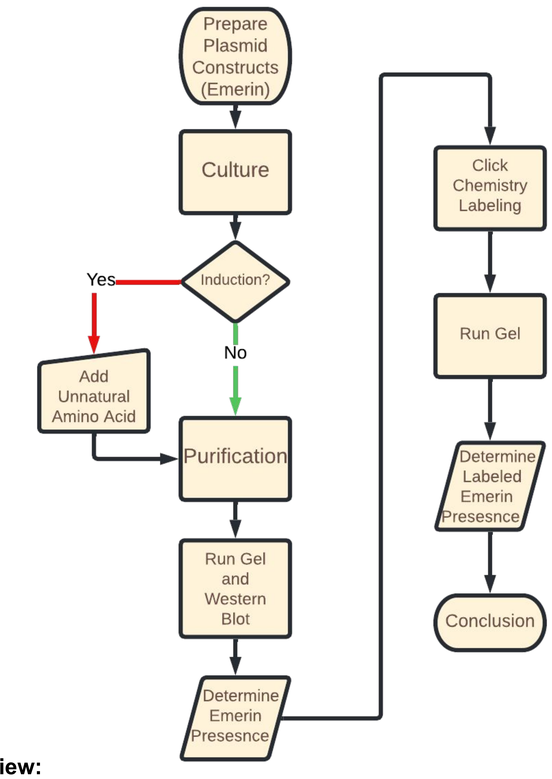
- The objective of this project is to develop and test an Emerin negative controls for future research in studying Emerin/Emerin interactions.
- This was done through the withholding of the unnatural amino acid necessary for the labeling as well as the production of emerin while transforming the E. Coli.

BACKGROUND INFORMATION



- Emerin is a protein that regulates nuclear structure by binding with other emerin and similar proteins.
- The lack of emerin is linked to Emery-Dreifuss muscular dystrophy. To understand how emerin takes part in molecular scaffolding, Emerin to Emerin interactions are currently being studied.
- Emerin/Emerin Interactions are studied via nano imaging techniques observing the tagged LEM domains of the Emerin.
- Click Chemistry labeling is a technique for site specific labeling. This is used to tag the LEM domain by adding an unnatural amino acid during Emerin production.
- This project is creating a negative control withholding of the unnatural amino acid necessary for the labeling as well as the production of emerin while transforming the E. Coli.

METHODS



Methods Overview:

Culture and induction:

This step is to produce Emerin in a E. Coli culture and to add the unnatural amino acids for the later click chemistry labeling. In this project the unnatural amino acid is withheld to create a negative control. The unnatural amino acid, OPGY UAA, allows for the emerin code to be read and produced, and later serves as docking for the site specific fluorescent labeling on the LEM domain. Without the unnatural amino acid, no emerin should be produced, and no emerin should be tagged. The general steps in this section are-

Purification and Extraction:

This section extracts emerin and various other proteins from the E. Coli solution previously prepared, then is filtered to have a emerin stock. This is done by using a IMAC binding column to pull all the proteins with a his-tag from the protein solution. The IMAC column contains Nickel beads known for binding to his-tags, and the Emerin contains a 10x His-tag. After the protein solution is put through the column. The flowthrough contains all non his-tag containing proteins. With a few diluted washes of imidazole the non-specific binding and proteins other than emerin are removed. The final emerin solution is dialyzed to get the negative control.

Click Chemistry Labeling:

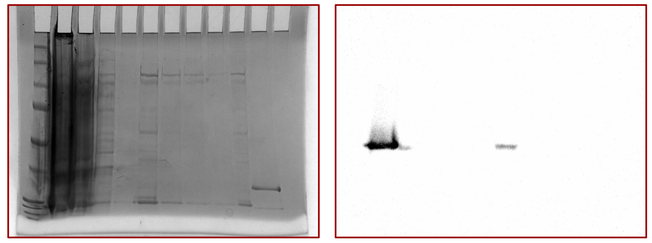
The Click Chemistry Labeling targets the previously placed unnatural amino acids in the emerin to tag with the Alexa 647-Azide dye.

Data Collection:

The data for this project is mainly acquired through protein gel electrophoresis, western blots, and their imaging. Samples from each step of the process run through a gel and western blotted to detect the presence of the lack of Emerin. A final gel is run with a positive control and a sample of the post click chemistry emerin solution to see if there is any labeled Emerin in the prepared negative control.

RESULTS

Emerin Presence Gel & Western Results



The Gel and Western Blot show the faint presence of emerin in the first elution fraction. This is likely due to codon read throughs.

Emerin Click Chemistry Labeling Results

A click chemistry labeling was done on a positive control and the first elution fraction that showed the presence of emerin. No labeled emerin was found on the gel after fluorescent gel imaging.

SUMMARY AND CONCLUSION

- The negative control was found to have some Emerin due to leaky termination.
- The Emerin was devoid of any unnatural amino acids which resulted in no fluorescent labeling of the remaining emerin in the negative control
- The production of the negative control was largely successful, however some issues should be addressed in the future including, the oligomerization of the protein, nonspecific binding, and further optimization in the purification of Emerin.

REFERENCES

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CONTACT US

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