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Modification of Aminoacyl tRNA Synthetase in Order to Incorporate An Unnatural Amino Acid

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Abstract

Proteins allow daily processes in the cell to occur. A protein consists of amino acids. There are twenty natural amino acids coded for in the DNA of organisms. The natural amino acids can be modified to form unnatural amino acids (UAA). UAs have useful characteristics when inserted into a protein, like the ability to fluoresce and the ability to undergo unique reactions. For an UAA to be incorporated into a protein, it must be bound to a transport RNA molecule by an enzyme called aminoacyl tRNA synthetase (aaRS).

Background

Figure 1. Structure of 3-(2-pyridyl)-L-Alanine (PyAla). Because of its structure, it has the capability to bind metals, specifically Cu2+ (2).

Figure 2. Visualization of the process for how a tRNA molecule gets charged with an amino acid.

Methods

The goal of this project is to mutate the aaRS so it will incorporate PyAla using directed evolution. After mutations, the aaRS must undergo cage screens to determine its effectiveness. The positive screen tests for PyAla incorporation, and the negative screen tests for PyAla specificity. The positive and negative screen utilize the amber suppression method of unnatural amino acid incorporation, shown in Figure 3. A plasmid coding for a 150TAG mutated green fluorescent protein (sfGFP 150) was inserted into E. coli cells containing the mutated aaRS and TAG tRNA plasmid.

Figure 3. A TAG (stop) codon is inserted into the middle of the DNA sequence (sfGFP) that codes for the protein to be expressed. If the aaRS does not work, and there is no UAA-charged tRNA to override the stop codon, the full length protein is not expressed (no fluorescence). However, if the aaRS does bind the tRNA and UAA together, the TAG stop codon will be overridden, the full length protein will be expressed, and the cells will fluoresce green.

The results of the positive screen (Figure 5) on all of the mutated aaRS plasmids.

Conclusions and Future Work

• Only aaRS mutation 4 solely incorporated the UAA of choice, PyAla, while all other mutations 2, 5, 6, 7, 8, and 9 incorporated both PyAla and PCNF, indicating those mutation(s) imparted increased permselectivity towards UAA incorporation.

• Future work will include confirming mutation sites with sequencing analysis, expanding the plasmid library via additional saturation mutagenesis, and performing the negative screen on positive hits (4) using a mutated barnase gene containing plasmid.

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Peter Schultz at Scripps Research Institute, Scott Gradia at Berkeley, Robert Hartley for the gift of their plasmids from AddGene deposition, and Ryan Mehl at Oregon State University for sharing his plasmids.

References

3) Khan Academy. Polymerase Chain Reaction (PCR).

Figure 4. Visual representation of the possible mutation points in the original pULTRA-CNFAaRS enzyme.

The pULTRA plasmid containing the genes for the p-CNFAaRS and TAG-tRNA was mutated via saturation mutagenesis and degenerate primers for positions L32, V65, W108, G158, and A159 using PCR. (3) The mutated plasmids were transformed into E. coli cells and grown in spectromycin resistant media. The purified plasmid DNA was restricted on agarose gel, and then cells were co-transformed with the plasmid containing the sfGFP 150TAG gene and run through a positive screen. For the positive screen, cells were grown overnight in a culture tube, then 1.5 mL of the culture was added to a flask with spectomycin and ampicillin antibiotics for selection. The cells were incubated for 1.5 hours, and PyAla (25 mM) was added to one flask, PCNF (25 mM) was added to the second, and no UAA was added to the third flask.

Figure 5. Graphs of the results of the positive screen on the aaRS mutations. The first column shows the control, which has no UAA's added to the broth. The middle column shows the fluorescence of the plasmid with PyAla added, and the third is with PCNF added. Mutant 4 (green box) indicated is the positive hit.

Figure 6. The three flasks used for the positive screen of co-transformed library plasmid 5. The first flask solution was not glowing green, which is expected, as no UAA was added. The second flask solution that had PyAla added was not glowing, which meant the plasmid failed the positive screen. The third flask solution was glowing, meaning PCNF was incorporated.