Green Fluorescent Protein as a Marker to Examine a Cell-Free System

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During the past few decades, in vivo techniques of cloning have been used to produce and examine proteins, and to study the mechanisms of protein synthesis within a cell. However, in vivo techniques are not practical for all applications, and a cell-free system may be more practical for certain circumstances. In vitro systems are useful for determining the mechanisms of producing a certain protein, as it is much easier to manipulate conditions such as the concentration of certain protein translational machinery in the extract, temperature, pH, or ionic strength, when the microenvironment of a living organism does not have to be contended with. Cell-free systems are also useful for producing proteins that may be toxic to a host organism, or require conditions that would harm the host, such as high temperatures, extreme pH values, or strong reductive/oxidative states. In vitro systems are also needed for site-specific incorporation of unnatural amino acids. A major disadvantage of cell-free systems is their tendency to have a low efficiency of protein production, sometimes so low that the protein cannot be detected. Improvements have been made on cell-free systems to increase their efficiencies, including the incorporation of a semi-permeable membrane, and a continuous flow of cell extract.

In order to test the efficiency of a cell-free system, it is best to use a protein that can easily be detected, even in low quantities. One protein that may be useful in determining the efficiency of such a system is green fluorescent protein (GFP). GFP contains 238 amino acids, and three of these, serine 65, tyrosine 66, and glycine 67, undergo a post-translational autocyclization reaction to produce a chromophore. This chromophore causes GFP to absorb UV light, and emit green light. As GFP does not require substrates or cofactors to fluoresce, it is relatively simple to determine the amount of protein produced by a cell-free system using fluorescence. GFP is a fairly stable protein, remaining fluorescent in temperatures up to 70°C, pH values ranging from 6-12, and in the presence of certain detergents.

A cell-free system was tested with two variants of GFP. Each variant was in a different plasmid, one with an arabinose promoter, and the other with a lacZ promoter. The GFP was purified, and the photophysical properties were compared, including the absorbance and emission spectra, and extinction coefficients. The plasmids containing the two variants of GFP were purified, and used to test the cell-free system.