44c An in Vitro Expression Method for the Production and Study of Iron-Sulfur Proteins

Marcus E. Boyer, James A. Stapleton, Chia-wei Wang, and James R. Swartz

Iron-sulfur (Fe-S) proteins constitute an important class of biological molecules involved in such processes as electron transport, redox and non-redox catalysis, and regulatory sensing. Among the most interesting examples of Fe-S proteins are hydrogenases, which catalyze the reduction of protons into hydrogen gas. Currently, the bulk of efforts toward sustainable hydrogen production by biological means involve hydrogen generation using hydrogenase enzymes. We have employed a cell-free system based on extracts of *Escherichia coli* to simultaneously transcribe, translate, and mature Fe-S proteins, beginning with the model Fe-S protein, ferredoxin from *Synechocystis* PCC 6803. This work has been conducted as a developmental tool for the production and screening of hydrogenase proteins. As an *in vitro* translation system, many parameters can be optimized in the cell-free system to maximize the yield of active Fe-S proteins. Among those studied, the supplementation of iron and sulfur sources in the form of ferrous ammonium sulfate and cysteine is essential. Optimum results have been obtained in supplemented concentrations far above those possible *in vivo*. Variations on reaction temperature show that maturation is favored at lower temperatures. Fe-S protein production has been accomplished in both aerobic and anaerobic environments. In addition to non-biological factors influencing protein production and maturation, enzymatic folding factors can be introduced into the cell-free system. This can be accomplished by the addition of purified factors, by the expression of folding factors in the *E. coli* strain used for cell-free extract, or as a coexpression product during a cell-free expression experiment. We have focused on the expression within the extract strain of folding factors known to assist in the maturation of Fe-S proteins. The effect of these helper proteins on the production and maturation of Fe-S proteins in cell-free extracts has been assessed. As a platform for Fe-S protein study, this system offers several advantages. We have shown it to be robust and adaptable. It is readily scalable to allow both high-throughput experiments and larger-scale production for purification of protein samples. It is efficient, with achieved active protein yields on the order of hundreds of micrograms per milliliter of reaction. As an open translation/maturation system, many methods are available for the detection, labeling, quantification, and assaying of the protein product under study. Protein mutants can be generated and screened starting with linear or circular DNA libraries. This cell-free system is currently being extended to the production of hydrogenase enzymes.