299g Improved Membrane Protein Expression Using Cell-Free Protein Synthesis

Jessica J. Wuu and James R. Swartz

Membrane proteins perform many critical functions in living systems, from transporting materials across cell boundaries to roles in cell adhesion and signaling. Their importance makes them popular targets for research and drug development. However, the study of membrane proteins has been hampered by generally low yields obtained from *in vivo* expression due to cell toxicity. To overcome this obstacle, we have developed an *in vitro* approach for producing membrane proteins using cell-free protein synthesis. Our cell-free system uses *E.coli* derived extracts to provide the enzymatic machinery needed for protein expression. Upon addition of a DNA template, we are able to recreate the transcription and translation reactions in an open environment that can be easily monitored and manipulated. By adding vesicles derived from the inner membrane of *E.coli*, we can also provide the proper environment and machinery needed for membrane protein insertion and folding. To demonstrate our technique, we have expressed two *E.coli* transporters, mannitol permease (MtlA) and the tetracycline pump (TetA). Both MtlA and TetA are integral membrane proteins containing six and twelve transmembrane segments, respectively. MtlA is known to depend on the SRP/FtsY pathway for insertion, while TetA is also suspected to use the same pathway. Both proteins express well in our system, greater than 700□g/mL, with a significant fraction inserted into vesicles as determined by Proteinase K digestion assays. Proper folding was also verified by assaying for transport activity. These yields, which are 10 to 100 times greater than those commonly obtained *in vivo*, demonstrate the potential for cell-free technology to significantly facilitate the further study of membrane proteins.