Chapter 24

Large Complexes: Cloning Strategy, Production, and Purification

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Abstract

Membrane proteins can assemble and form complexes in the cell envelope. In Gram-negative bacteria, a number of multiprotein complexes, including secretion systems, efflux pumps, molecular motors, and pilus assembly machines, comprise proteins from the inner and outer membranes. Besides the structures of isolated soluble domains, only a few atomic structures of these assembled molecular machines have been elucidated. To better understand the function and to solve the structure of protein complexes, it is thus necessary to design dedicated production and purification processes. Here we present cloning procedures to overproduce membrane proteins into *Escherichia coli* cells and describe the cloning and purification strategy for the Type VI secretion TssJLM membrane complex.

Key words Membrane protein complexes, *Escherichia coli*, T7 overexpression, Protein purification

1 Introduction

Protein overproduction results from cloning a gene of interest into a plasmid vector, downstream of a tightly regulated promoter, and from inducing its expression after plasmid transformation into a bacterial strain. For large protein complexes containing multiple subunits, the genes encoding the different subunits can be expressed under the control of an inducible promoter either from a single plasmid containing a cluster of genes or from different compatible plasmids harboring single or multiple genes.

1.1 Cloning Vectors

Several inducible promoters have been described and are available to overexpress a gene of interest. These promoters are usually cloned into vectors that also contain the gene encoding the cognate regulatory protein and a transcriptional terminator to prevent non-productive transcription from the downstream gene [1–7]. The *tac* and *trc* promoters that contain the −35 and −10 sequences from the *trp* and *lacUV5* promoters, respectively, have been optimized for high expression levels [1]. The most famous regulated *E. coli*

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