PRODUCTION OF MEMBRANE PROTEINS IN THE FORM OF TUBULAR STRUCTURES

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Introduction

A barrier in the determination of membrane protein structures is sample preparation, which usually requires detergent solubilization, removal of the lipid and either maintaining the specimen in a monomeric state for solution NMR, or organizing it into crystalline arrays for X-ray crystallography or solid state NMR. The approach described here obviates these challenges. The tubular structures are potential candidates for atomic resolution characterization by cryoEM if well enough ordered, and if the helical structures are relatively homogeneous. In addition, tubular preparations may be excellent samples for SSNMR if the protein structure is homogeneous throughout the sample. Proteins need not be expressed with purification tags as the isolation of the tubes is sufficient purification. Since the tubes are formed by proliferated membranes at the time of protein induction nearly all of the membrane protein in the tubes is the desired protein. If the protein is inserted into the tubes in a native conformation, the samples for structural characterization in the isolated tubes will not be exposed to detergent at any stage of the sample preparation.

Experimental

For Electron Microscopy, E. coli cells harboring membrane proteins from tuberculosis were grown in LB media at 37 °C. Protein was induced with IPTG for 3 hours. For isotope labeling, harvested cells from 4 liters of LB media were washed once in M9 media and then inoculated into 1 liter of M9 media. Tubules were isolated by sucrose gradient centrifugation.

Mycobacterium tuberculosis membrane proteins over-expressed in E. coli were surveyed on glow discharged, carbon-coated grids and negatively stained with 2% Uranyl Acetate. A Phillips CM120 Electron microscope at 80kV was used.

Results and Discussion

The bacterial host responds to the over production of protein by increasing the amount of phospholipid in the cytoplasmic membrane, thus keeping the lipid/protein ratio relatively constant and then organizing the excess lipid and protein into these ordered tubular arrays. Of 65 clones tested, 12 formed some degree of tubular structures. Rv2433, had the most abundant population of tubes observed within the cell (Figure 1). These tubes run the length of the cell following along the cytoplasmic membrane and congregate as bundles instead of individual units. We have optimized the isolation experiments on the Rv2433c tubes. Tube production has been achieved for this protein in defined M9 medium.

Figure 1: Negative-stain electron microscopic cross-section of Rv2433c over-expressed in E. coli (bar= 100nm).

Conclusions

Our work so far has indicated that just on testing of high expressing clones, that about 20% show evidence of tubular specimen at some level. Without any optimization, ~6% show high levels of expression. About 50+% produced structures that might be useful for structure work with further optimization. Since there are about 1,100 putative membrane proteins in M. tuberculosi s genome, this should yield a large number of specimens suitable for cryoEM. Thus, this approach shows high probability of success and provides a high throughput methodology for cryoEM to contribute to membrane protein structural genomics.

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