

The Photosynthetic Bacterium *Rhodobacter capsulatus* as an Alternative Platform Organism for the Expression of Human Membrane Proteins

Armagan Özgür

Katrin Pünder; Achim Heck; Karl-Erich Jaeger; Martin Gamer[*]; Thomas Drepper

Heinrich-Heine University, Institute of Molecular Enzyme Technology, Forschungszentrum Jülich, Jülich, 52425 Germany

[*] Protagen AG, Otto-Hahn Strasse 15, Dortmund, D-44227 Germany

Heterologous expression of human membrane proteins is a major concern for medicinal and pharmaceutical research due to the fact that nearly 50% of available drugs are either directly or indirectly targeting human membrane proteins. However, the intricate nature of membrane proteins hampers their structural and functional studies because common expression hosts like *E. coli* are optimized for the production of soluble proteins. Therefore, we developed a new expression system based on the facultative phototrophic non-sulfur purple bacterium *Rhodobacter capsulatus*. During phototrophic growth, *R. capsulatus* forms a continuous system of intracytoplasmic membranes (ICM) harboring the integral membrane protein complexes of its photosystem. Thus, due to its phototrophic nature, *R. capsulatus* is perfectly adapted to synthesize membrane proteins by providing a highly enlarged membrane surface and an efficient membrane protein folding and translocation machinery. To allow a concerted induction of heterologous membrane protein expression and ICM formation, we first constructed a new set of expression plasmids that allows the gradual expression of target genes in the bacterium under phototrophic growth conditions. To evaluate the applicability of the novel expression system, several human proteins that are either membrane-associated or integral membrane proteins exhibiting 1 to 7 transmembrane helices (TMHs), were comparatively expressed in *E. coli* and *R. capsulatus*. Protein accumulation and localization studies revealed that *E. coli* seems to be the preferable expression host for membrane proteins with a low number of TMHs, whereas membrane proteins with a higher number of transmembrane domains achieve higher protein yields with the newly developed *R. capsulatus* expression system. Therefore, the photosynthetic bacterium *R. capsulatus* is a promising alternative platform organism for the heterologous expression of more complex membrane proteins.