

Bacteriorhodopsin Biogenesis: from Polypeptide to Membrane Crystal.

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The integral membrane protein bacteriorhodopsin (BR) is the first-discovered member of a superfamily of microbial rhodopsins that function as light-driven ion pumps and phototaxis sensors in archaea, bacteria and eukaryotes. Because its structure is known at atomic resolution, BR is attractive for studying events in integral membrane protein biogenesis, including insertion into the membrane, folding and assembly into multimeric structures. BR consists of a polypeptide (bacterioopsin) containing seven transmembrane α -helices that provide a binding pocket for vitamin A retinal, which is covalently bound to the molecule and is responsible for its purple color. In *Halobacterium salinarum*, which produces BR, the protein assembles with unique membrane lipids in extensive two-dimensional crystals known as the purple membrane.

We seek to understand each step in BR biogenesis at the molecular level. In earlier work, we demonstrated that bacterioopsin is inserted into the *H. salinarum* membrane as it is translated. External loops of the protein appear outside the cell in the same order as they occur in the primary sequence, in accord with current models of membrane protein integration. We also identified genes that are essential for retinal production in *H. salinarum*, including some that are related to known carotenoid biosynthetic genes and others that may be specifically required for the assembly of retinal with the polypeptide chain of microbial rhodopsins. Finally, we showed that specific amino acid residues in transmembrane α -helices contribute to purple membrane assembly and stability.

More recently, we used semi-random mutagenesis in *H. salinarum* to identify the structural features of BR that determine its biogenesis. We targeted substitutions to regions of ~15 amino acids throughout the protein with a doped oligonucleotide PCR strategy designed to yield an average of five substitutions in each mutant protein. The sequence of the bacterioopsin gene in 873 unique mutants that yielded purple colonies indicated that most residues could be altered with little effect on BR formation. Eight of the 238 residues targeted were unaltered in any mutant and ~15 residues tolerated only conservative changes. Most of these residues are in the retinal binding site where mutations are known to influence BR formation and the color of the protein. However, several residues outside of the retinal binding pocket were identified that may be essential for BR formation. We conclude that BR insertion and folding is generally independent of the amino acid sequence except in the retinal binding pocket. The implications of these results for membrane protein biogenesis will be discussed.