

**ESCHERICHIA COLI AS A MODEL IN TARGETING THYLAKOID PROTEIN, CYTOCHROME  $b_6$ , INTO THE MEMBRANE**

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Chloroplast contains at least four distinct pathways for targeting proteins to the thylakoid membrane [1]. All of these, the Sec-dependent, SRP-dependent,  $\Delta$ pH-dependent and spontaneous pathways have direct cognates in bacteria [2, 3]. Observed similarity of these mechanisms are consistent with the evolution of the chloroplast from a prokaryotic ancestor. Several thylakoid signal peptides can target protein for secretion across the plasma membrane of bacteria [4, 5]. Usually after overexpression in *Escherichia coli* membrane protein its accumulates in bacteria as inclusion bodies [6, 7], however integration of some thylakoid membrane proteins (cytochrome *f* and LHCP) into *Escherichia coli* cytoplasmic membrane was also shown [8, 9]. Since membrane proteins contain domains that must be translocated across the bilayer, it is no surprise that the enzymatic machinery responsible for translocating proteins across the membrane also plays a role during the assembly of integral membrane proteins. An important notion is that the insertion machinery may not only insert the membrane protein but also determine its topology. Only when insertion machinery is able to understand the "language" used by the polypeptide chain, the polypeptide will have the native structure in the membrane.

Chloroplast encoded cytochrome  $b_6$  like many other integral proteins operate with uncleaved signal for insertion into thylakoid membrane. To determine whether cytochrome  $b_6$  may be inserted into bacterial membrane *Spinacia oleracea* gene *petB* was fused:

- (i) to the *pelB* signal sequence, directing to the SecA dependent pathway, plasmid pET25b(+) (Novagen) resulting plasmid pET25bb<sub>6</sub>;
- (ii) to the *Escherichia coli* hydrogenase 1 signal sequence and to the *Escherichia coli* hydrogenase 1 small subunit directing to the TAT pathway resulting plasmids psHb<sub>6</sub> and pHyab<sub>6</sub>;
- (iii) gene *petB* was also cloned to the plasmid pET16b in polylinker site without any additional signal sequences, resulting plasmid pET16bb<sub>6</sub>.

Plasmids were expressed in *Escherichia coli*, strain BL21(DE3). Expression product apocytochrome  $b_6$  fused to the C-terminus of the *pelB* signal peptide was incorporated into the membrane. After overexpression of pET16bb<sub>6</sub> we found apocytochrome  $b_6$  protein in inclusion bodies and weak signal in the inner membrane, moreover during overexpression apocytochrome  $b_6$  was degraded. After expression in presence of hemin only cytochrome  $b_6$  fused to the *pelB*

sequence gave maximum absorbance at 432 nm suggesting presence of minimum one heme and proper insertion into bacterial membrane.

Two fusion proteins: cytochrome  $b_6$  fused to the hydrogenase 1 signal sequence and to the precursor of hydrogenase 1 small subunit, were localized in inclusion bodies but not in the membrane.

Next step in our research it is to clone *petB* gene to the fragment of *Escherichia coli* gene *lep*, encoding leader peptidase I, which will direct fused cytochrome to the membrane through the SRP way [10].

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