THE LOCALISATION OF PhoE and β-GALACTOSIDASE ANTIGENS IN WILD TYPE AND PhoE/LacZ HYBRID *Escherichia coli*

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PhoE, one of the outer membrane proteins in *E. coli* K-12 cells, is involved in the uptake of small hydrophilic, especially negatively charged solutes. Its synthesis is induced upon phosphate limitation. Several models have been proposed for the mechanism of transport and incorporation of outer membrane proteins into the outer membrane (1). *Escherichia coli* cells containing a hybrid phoE/lacZ gene were studied to investigate the destination of the hybrid gene product, using an immunocytochemical labeling procedure and ultra-thin cryosectioning.

In cryosections of wild type *E. coli* K-12 cells the use of monoclonal antibodies against phoE trimer resulted in a dense labeling of the outer membrane, whereas β-galactosidase was found randomly distributed throughout the cytoplasm (Fig. 1). The use of polyclonal antibodies against monomeric phoE resulted in a weak labeling which was randomly found over the sectioned bacteria.

Upon induction of cells containing the hybrid phoE/lacZ gene, monomeric phoE and β-galactosidase antigens were found in the cytoplasm in electron dense areas which seem to be closely associated with the cytoplasmic membrane (Fig. 2). The periplasm and the outer membrane were only slightly labeled. Weak labeling was found both in the cytoplasm and in the outer membrane when monoclonal antibodies against the trimeric form were applied. Our results suggest that the hybrid phoE/lacZ gene product is not incorporated in the outer membrane, but is retained in the cytoplasm, probably in close association with the inner membrane.

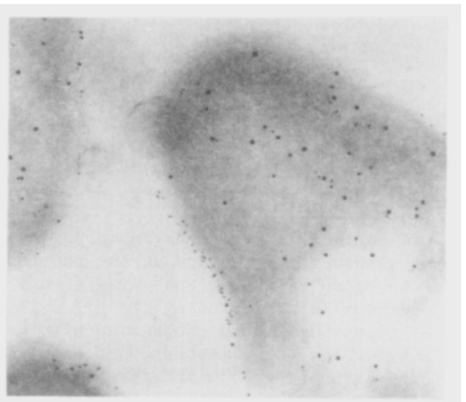

Fig. 1: *E. coli* pJP14 ("wild type"), double-labeled for phoE (trimer) with protein-A/gold (ϕ 8 nm) and β-galactosidase with protein-A/gold (ϕ 12 nm).

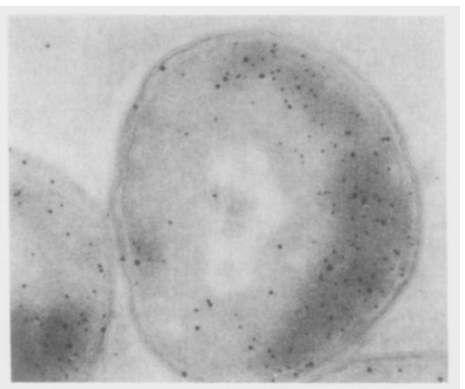

Fig. 2. *E. coli* pJP102 ("hybrid"), double-labeled for phoE (monomer) with protein-A/gold (ϕ 12 nm) and β-galactosidase with protein-A/gold (ϕ 8 nm).

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REFERENCE

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