Mobilizable bacterial DNA packaged into membrane vesicles induces serial transduction

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Experiments where the amino acid deficient strain *E. coli* AB1157 was exposed to a particle fraction harvested from a marine oligotrophic environment, ranging in diameter size between 100–130 nm indicated evidence for horizontal gene transfer resulting in revertant cells with restoration of all genetic deficiencies with frequencies up to 1.94×10^{-5} . None of the markers was preferentially transferred indicating that the DNA-transfer is performed by generalized transduction. The highest transfer frequency obtained for single markers was 1.04×10^{-2} . All revertant strains were able to produce particles of comparable size that were again infectious, appearing at the beginning of the stationary phase. Ultra structural investigation showed a structural resemblance with membrane vesicles, however, Field Gel Electrophoresis indicated that the DNA content of some of the particles was 370 kbp; much higher than that of the so far known previously described membrane vesicles providing evidence of a new mechanism for horizontal gene transfer.

Key words: virus-like particles, membrane vesicles, marine bacteria, horizontal gene transfer (HGT), serial transduction

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uptake of free DNA from the environment, transformation, (2) the transfer of DNA from a bacterial donor cell to a bacterial recipient cell termed conjugation, (3) the phage-mediated passage of bacterial genes referred to as transduction, (4) the gene transfer agents (GTA), an unusual bacteriophage-like vehicle of genetic exchange. We want to draw attention to a fifth mechanism, the transfer of DNA packaged in membrane vesicles which are formed by Gram negative bacteria.²⁻⁸ Because spontaneous formation of membrane vesicle-like particles by bacteria in the stationary was observed in earlier studies, we sampled the particle size class $<0.2 \mu\text{m}$ from the Mediterranean Sea, separated the 100–130 nm size fraction by CsCl-density equilibrium ultracentrifugation and performed gene transfer experiments with an auxotrophic mutant strain *E. coli* AB1157. Growth studies of revertant cells, ultrastructural characterization and DNA-antibody labeling of TEM thin sections completed the gene transfer assays. Remarkably high gene transfer frequencies up to 10^{-2} were obtained for single markers.

One of the most surprising features of this study is that the results of this gene transfer are revertant cells, which are able to produce new particles which are released by budding (Fig. 1). These particles produced by the first cell generation (F1) are again infectious and possess also the capability to transfer genes. The resulting second cell generation (F2) was observed to manifest again particle production. These particles had a mean diameter and mode similar to those produced by the F1 cells, were again infectious

Prokaryotes are known to be unique in their ability to react to environmental changes by the rapid acquisition of the necessary and appropriate genetic traits. This genetic flexibility of the prokaryotes is mainly due to the natural existence of seemingly efficient means for horizontal gene transfer among bacteria as well as between bacteria and other organisms.¹ Genetic exchange, therefore, plays a key role in the evolution of prokaryotes and the mechanisms responsible for the horizontal DNA transfer include (1) the

Figure 1. Ultrathin section of a transduced bacterial cell (*E. coli* AB1157) in the process of budding. EDB: Electron dense bodies, EDN: Electron dense network.

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and induced the production of an F3 revertant population, a phenomenon we termed serial transduction. The investigated particles show analogy to classical membrane vesicles but transfer contain large amounts of DNA up to 370 kbp to

the recipient cells and are larger than so far known viruses, GTA particles or membrane vesicles. Recent genome analyses of membrane vesicles derived from two different Alpha-Proteobacteria revealed the predominance of bacterial sequences.