INTERACTION BETWEEN ISOLATED PHAGE λ DNA AND *Escherichia coli* CELL MEMBRANE STRUCTURES

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The successful use of frozen and thawed bacteria and of bacteria treated with Ca^{++} cations in the cold as recipients of isolated phage, plasmid, and chromosomal DNA has brought with it the need to elucidate the mechanisms forming this competence in cells. Little is yet known of the nature of these processes, and discussion centers particularly on the possible contribution of transient reparable injuries to the cell membrane, in the case of low-temperature treatment of bacteria, and subsequent exposure of the recipient at positive temperatures, to the formation of competence [1, 6]. There is no information about the mechanism forming competence in bacteria treated with $CaCl_2$ solution. It is known that Ca^{++} ions cause conformational changes on the outer side of cell membranes [4] and that Ca^{++}-dependent DNA uptake is energy-independent [3].

The object of this investigation was to study interaction between isolated phage λ DNA and the membrane structures of *Escherichia coli* cells when treated with Ca^{++} cations in the cold and when these bacteria are frozen and thawed together with phage DNA.

EXPERIMENTAL METHOD

DNA-^{3}H of phage λC1857, obtained by thermoinduction of *E. coli* CR-34 (λC1857/B_1, tre, leu, thy; obtained from Beckwith, USA) was used for transfection as described previously [2]. *E. coli* strain Hfr H (B_1, λ^-, S^S), was used as the recipient. The cells were treated with Ca^{++} ions by the method of Mandel and Higa [7], using 0.1M $CaCl_2$. Combined freezing of bacteria and phage λ DNA was carried out at −196°C in liquid nitrogen by a one-stage program, with freezing at the rate of 400°C/min. The samples were kept at −196°C for 3 min and then thawed to 42°C at the rate of 150-200°C/min.

The DNA-membrane complex was isolated from infected cells by mild lysis, using the detergent Brij-58 [5]. The membrane fractions were separated by centrifugation in a 30-55% sucrose gradient on a Spinco centrifuge with SW-39 rotor for 16 h at 35,000 rpm. Samples of 0.3 ml were taken from the fractions and their density determined with a refractometer. Radiometry of the samples was carried out on a Beckman liquid scintillation counter. To determine radioactivity on the filters standard scintillation fluid (0.6% PPO and 0.01% POPOP in toluene) was used.

EXPERIMENTAL RESULTS

The main aims of the investigation were, first, to detect possible differences in the character of distribution of phage DNA among the membrane structures of *E. coli* cells treated with Ca^{++} cations in the cold and frozen and thawed cells compared with untreated cells; second, to assess quantitatively the efficiency of binding of phage λ DNA with the cell wall and cytoplasmic membrane of the recipients; and third, to analyze the experimental data from the point of view of their influence on transfection efficiency.

It was shown that the greater part of the radioactivity corresponding to phage DNA on intact *E. coli* cells was located in the fraction with a density of 1.22 g/ml, characteristic of the cell wall (Fig. 1a). During treatment of the cells with Ca^{++} cations in the cold, redistribution of the phage DNA from the cell wall to the cytoplasmic membrane took place (Fig. 1b). In the case of freezing and thawing of the cells together with infectious DNA, a uniform distribution of phage DNA was observed among fractions with a density of 1.22 g/ml,

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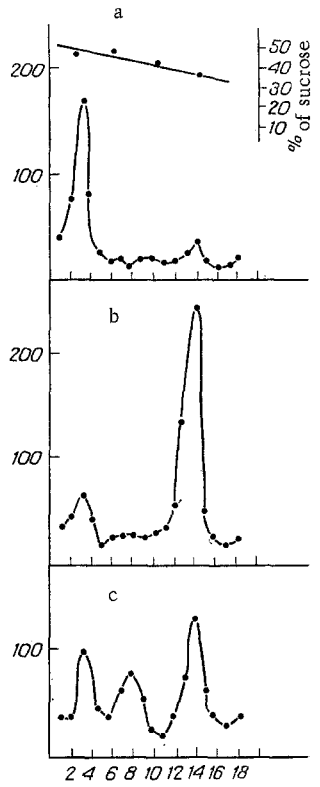

Fig. 1. Distribution of phage λDNA-^{3}H among membrane fractions of E. coli. a) Intact bacteria; b) bacteria treated with $CaCl_2$ solution; c) frozen and thawed bacteria. Abscissa, fraction nos.; ordinate, radioactivity (in CPM).

TABLE 1. Distribution of Phage λ DNA among Membrane Fractions of E. coli Cells (in % of DNA adsorbed on bacteria; M ± m)

Experimental conditions	cell wall	cytoplasmic membrane	Intermediate fraction
Intact bacteria	57,5±7,5	7,0±1,5	3,5±0,5
Bacteria treated with $CaCl_2$ solution	10,8±2,5	38,0±3,2	8,0±0,9
Frozen and thawed bacteria	16,7±2,3	17,7±5,3	17,3±3,2

characteristic of the cell wall, 1.14-1.6 g/ml, characteristic of the cytoplasmic membrane, and 1.18-1.19 g/ml, characteristic of the intermediate fraction (Fig. 1c).

Quantitative estimation of the distribution of phage DNA among the membrane fractions of the recipients (Table 1) showed that for intact cells 50-60% of the quantity of added DNA adsorbed by the cell was bound with the cell wall. For cells treated with Ca^{++} cations about 40% of the adsorbed DNA was bound with the cytoplasmic membrane and only 10% with the cell wall. As regards the frozen and thawed cells, on average, 17% of adsorbed DNA was bound with the cell wall, the same amount with the cytoplasmic membrane, and the same amount was found in the intermediate fraction.

Analysis of the sedimentation diagrams led to the conclusion that the mechanism of uptake of phage DNA by the frozen and thawed bacteria was different from that of their uptake by cells treated with Ca^{++} cations in the cold. Preliminary treatment of the cells with Ca^{++} ions in the cold evidently causes some of the cations to be transported through the cell membrane, and this facilitates subsequent penetration of infectious DNA and its attachment to the cytoplasmic membrane. This may perhaps explain the large quantity of phage DNA bound

with the cytoplasmic membrane of cells treated with Ca^{++} ions. Transient reparable defects in membrane structures of bacteria frozen and thawed together with phage DNA also promote the creation of conditions favoring DNA penetration. However, no preferential binding of transfecting DNA with the cytoplasmic membrane took place.

Investigation of interaction between phage λ DNA and membrane structures of cells treated with Ca^{++} cations in the cold and of frozen and thawed cells thus showed that procedures of this type lead to penetration of infectious DNA within the recipient, and that the mechanism of this penetration is different for each type of recipient. The binding of infectious DNA with the cytoplasmic membrane is evidently an essential condition for transfection; nevertheless, it is not sufficient by itself, for despite significant differences in the number of DNA molecules bound with the cytoplasmic membrane, the efficiency of transfection of these recipients is practically identical.

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