Transformation in *Escherichia coli*: Stages in the Process

H. E. N. BERGMANS,* I. M. VAN DIE, AND W. P. M. HOEKSTRA

Department of Molecular Cell Biology, State University of Utrecht, 3584 CH, Utrecht, The Netherlands

Received 30 October 1980/Accepted 11 February 1981

Transformation experiments with *Escherichia coli* recipient cells and linear chromosomal deoxyribonucleic acid (DNA) are reported. *E. coli* can be rendered competent for DNA uptake by a temperature shock (0°C → 42°C → 0°C) of the recipient cells in the presence of a high concentration of either Ca$^{2+}$ or Mg$^{2+}$ ions. Uptake of DNA into a deoxyribonucleic-acid-resistant form, for which the presence of Ca$^{2+}$ is essential, was possible during the temperature shock but appeared to occur most readily after the heat shock during incubation at 0°C. When DNA was added to cells that had been heat shocked in the presence of divalent cations only, DNA uptake also occurred. This suggests that competence induction and uptake may be regarded as separate stages. Under conditions used to induce competence, we observed an extensive release of periplasmic enzymes, probably reflecting membrane damage induced during development of competence. After the conversion of donor DNA into a deoxyribonucleic-acid-resistant form, transformants could be selected. It appeared that incubation, before plating, of the transformation mixture in a medium containing high Ca$^{2+}$ and Mg$^{2+}$ concentrations and supplemented with all growth requirements increased the transformation frequency. This incubation probably causes recovery of physiologically labile cells.

Uptake of free DNA by *Escherichia coli* can only be achieved when the recipient cells have been made "competent," either by turning them into spheroplasts (useful only in transfection, see reference 2) or by a heat shock in the presence of Ca$^{2+}$ ions, as first reported by Mandel and Higa (14). Several modifications of the latter method are now widely used for transformation of *E. coli* with plasmid DNA (4, 7, 11, 12). It has been shown that Ca$^{2+}$-treated cells can be transformed also with chromosomal DNA (17, 29). In that case, a significant number of transformants were obtained only when recipient cells were used that lacked recBC nuclelease activity and had been rendered recombination proficient by an sbc mutation. The fact that Rec$^−$ (recBC nuclease-proficient) cells yield very few transformants is probably due to effects that occur after DNA adsorption and uptake. There is genetic evidence that partial degradation of donor chromosomal DNA by recBC nuclease is responsible for the reduction of transformation frequencies in Rec$^−$ cells (9).

The original protocols for transformation of *E. coli* with chromosomal DNA (17, 29) essentially involved Ca$^{2+}$ pretreatment of *E. coli* cells at 0°C and heat shock of the cells in the presence of Ca$^{2+}$ and purified donor DNA. Transformants were subsequently selected on plates containing sodium citrate to bind excess Ca$^{2+}$ ions.

We recently described a modified procedure that yields a significantly higher transformation frequency (20). The main modifications are: (i) no extensive pretreatment of the cells with Ca$^{2+}$, (ii) the presence of Mg$^{2+}$ ions as well as Ca$^{2+}$ during the whole transformation procedure, and (iii) the use of selective plates that contain Ca$^{2+}$ and Mg$^{2+}$ ions, a low concentration of phosphate, and no sodium citrate. It is not clear why these modifications increase the transformation frequency. In this report we describe a series of experiments that give more insight into the complicated *E. coli* transformation system.

**MATERIALS AND METHODS**

**Bacteria.** All strains were *E. coli* K-12 derivatives. PC0031 is a prototrophic strain. AM1095 (*leu* pdaA ara car thi his trp recB21 recC22 sbcB15) is a transformable strain, described previously (10). AM1283 is a derivative of AM1170 (AM1095 rpoB) carrying plasmid R1'drd-19 (Ap$^R$) (1).

**Media and buffers.** Phosphate-based minimal salts medium was as described previously (30). In the Tris-based minimal salts medium, phosphate buffer was replaced by Tris-hydrochloride buffer (1.2 × 10$^{-1}$ M, pH 7.0). In morpholinopropanesulfonic acid (MOPS)-based minimal salts medium, the phosphate buffer was replaced by MOPS buffer (1.2 × 10$^{-1}$ M, pH 7.0). Media were solidified with 1.6% agar.

**Growth requirements** were added in the following concentrations (milligrams per liter): DL-leucine, 40; DL-arginine, 40; uracil, 20; pyridoxine, 10; DL-trypto-
VOL. MOPS-  
in a final concentration of 3 \times 10^{-4} M, was added to the medium.

DNA isolation. Donor DNA was isolated from strain PC0031 as described by Cosloy and Oishi (5). Routinely isolated DNA has a molecular weight of about 4 \times 10^8, as inferred from sedimentation on sucrose gradients with \lambda DNA as a reference.

Transformation procedure. The transformation procedure used is a modification of the procedure of Reijnders et al. (20). An overnight culture of the recipient cells in phosphate-buffered minimal salts medium was diluted 1:20 into fresh minimal medium and incubated with shaking at 37°C until the cell density measured in a Klett-Summerson photometer (650-nm filter) reached a value of 30. The culture was chilled, kept at 0°C for 15 min, washed once with 0.25 volume of 10 mM NaCl, and suspended in 0.05 volume of 20 mM HEPES (N-2-hydroxyethylpiperazine-N'-ethanesulfonic acid) buffer (pH 6.0) at 0°C. A portion (0.3 ml) of the cell suspension (10^9 viable cells) was mixed with 0.1 ml of donor DNA (final DNA concentration, 60 \mu g/ml), after which 0.1 ml of a solution containing 150 mM CaCl\(_2\) and 130 mM MgCl\(_2\) was added. The mixture was then carefully mixed on a vortex mixer and incubated for 10 min at 0°C, followed by 6 min at 42°C and 60 min (or a different period when indicated) at 0°C. When indicated, uptake of donor DNA was terminated by incubating the mixture with 400 \mu g of pancreatic DNase I (EC 3.1.21.1; Boehringer Mannheim Corp.) per ml for 30 min at 0°C. 

In some experiments, the transformation mixture was further incubated for 10 min at 37°C, or the transformation mixture was diluted 1:10 into MOPS-buffered minimal medium containing growth requirements and with CaCl\(_2\) and MgCl\(_2\) added as indicated. Portions of the transformation mixture were plated on selective Tris minimal medium supplemented with 0.01 M CaCl\(_2\) and 0.01 M MgCl\(_2\). In all experiments, Leu transformants were selected. The leu marker used reverted at a rate yielding less than 10^4 revertants per viable cell. For each sample from which transformants were selected, the number of viable cells was determined by plating suitable dilutions on tryptone agar plates. During several steps in the transformation procedure, there is a loss of viable cells. During the heat shock in the presence of Ca\(^{2+}\) and Mg\(^{2+}\) ions there is a 25% decrease. Incubation after the heat shock for 90 min at 0°C causes another 30% decrease. Transformation frequency was expressed as numbers of transformants per viable recipient cell.

\(\beta\)-Lactamase assay. Suspensions of AM1283 [AM1095 (R1rdrt-19 Ap')], at the same cell density as used in our transformation procedure contain a very high level of \(\beta\)-lactamase activity. For an assay using 33-\mu l samples and an incubation period of 5 min (see below), the cell suspension should be diluted 10-fold so as to contain a measurable enzyme activity. As we did not want to include additional dilution steps in the assay, but wanted to keep the cell density during the assays similar to that used in transformation experiments, we assayed \(\beta\)-lactamase activities in AM1283-AM1095 mixtures (1:10 ratio).

Cultures of AM1283 and AM1095 were grown separately, harvested, suspended in HEPES buffer in the same manner as in the transformation procedure and then mixed. Part of the mixed-cell suspension was converted into spheroplasts by a modification (13) of the EDTA-lysozyme method of Osborn et al. (18). \(\beta\)-Lactamase was assayed as described by Nikaido et al. (16). The reaction mixture (1 ml) contained 10 mM sodium phosphate buffer (pH 7.0), 0.8 mM ampicillin (Enzyfarm), and 33-\mu l samples from suspensions of whole cells, supernatant of these after centrifugation, or spheroplasts. The reaction occurred for 5 min at 28°C and was stopped by the addition of 1 ml of a solution containing 1 M acetic acid, 0.2 M sodium tungstate, 0.2% soluble starch, 0.18 M I\(_2\), and 7.2 M KI. After 20 min of incubation at 28°C, the absorbance at 623 nm was measured in a Bausch & Lomb Spectronic 700 spectrophotometer. A reaction mixture to which the "stopping" solution was added before the test sample served as a control for each incubation.

\(\beta\)-Lactamase activities are expressed in arbitrary units, being the difference in extinction at 623 nm between the control and test incubations.

\(\beta\)-Galactosidase assay. \(\beta\)-Galactosidase was assayed in toluene-treated samples of suspensions of whole cells or directly in samples of supernatants after centrifugation, essentially as described by Miller (15).

RESULTS

In our basic transformation procedure, saline-washed cells from an early-logarithmic culture of AM1095, a multiauxotrophic recB21 recC22 sbcB15 strain, were mixed at 0°C with DNA isolated from a prototrophic strain; CaCl\(_2\) and MgCl\(_2\) were added, and the mixture was shocked at 42°C for 6 min, followed by a 15-min incubation at 0°C. We observed that the frequency of Leu\textsuperscript{+} transformants was enhanced by a factor of 10 when the transformation mixture was plated on selective Tris-based minimal salts medium containing 10 mM Ca\(^{2+}\) and 10 mM Mg\(^{2+}\) instead of on conventional phosphate-based minimal medium. Since in this case uptake of DNA was not terminated with DNase, the observation could mean that transformation proceeds or may even start after the heat shock. Figure 1 and 2 show initial experiments which further elucidate the processes after the heat shock. In Fig. 1, the frequency of transformants resistant to DNase treatment is plotted versus the incubation time at 0°C after the heat shock. The DNase treatment if applied before the heat shock was sufficient to reduce the transformation frequency to less than 10\(^{-7}\) transformants per recipient cell. The results presented in Fig. 1 suggest that during incubation at 0°C, DNA was still converted into a DNase-resistant form, presumably by uptake of DNA into recipient cells rendered competent by the heat shock. Incubation of the transformation mixture at 0°C for additional
heat shock. When DNA formants shock, heat factor (lines A and B) served reducible. This additional effect was observed by stimulating DNase. In these experiments, the mixture was incubated at 42°C for 6 min and then further incubated at 0°C. Time zero is the time of transfer from 42 to 0°C. At times indicated, portions of the transformation mixture were incubated with DNase (30 min, 0°C), after which the frequency of Leu* transformants per viable cell was determined.

Periods longer than 180 min (up to 24 h) elicited no consistent increase in the yield of transformants. For practical purposes, we used an incubation period of 60 min at 0°C in further experiments.

If one postulates that transformation proceeds on suitable selective plates, it follows that plating of a transformation mixture implies one more heat shock, since the mixture kept at 0°C is then incubated at 37°C. Figure 2 illustrates the effect of a deliberately applied second heat shock 60 min after the first heat shock and before plating. This second heat shock was applied to mixtures that either had or had not been treated with DNase. In both cases, the second heat shock had a stimulating effect.

We cannot exclude stimulation of DNA uptake by the second heat shock when DNase treatment was omitted; however, the stimulating effect observed in the experiment with DNase treatment shows that there was at least an additional effect on some process(es) after the conversion of DNA to a DNase-resistant form. The stimulating effect was rather small but reproducible. In seven separate experiments, we observed such an effect. The average stimulation factor was 2.2 ± 0.6.

Aspects of DNA uptake. If DNA is taken up by competent cells in the period after the heat shock, it should be possible to obtain transformants when DNA is added to cells after the heat shock. The results presented in Table 1 (lines A and B) prove that indeed transformants were formed when DNA was added after the heat shock. The high transformation frequencies obtained when DNA was added after the heat shock clearly show that cells were rendered competent for DNA uptake by a heat shock in the absence of DNA. Lines C through I of Table 1 show further aspects of competence development. Without a heat shock, there was no detectable amount of transformants (line C). The presence of either Mg²⁺ or Ca²⁺ ions was required during the heat shock (lines E and F), since a heat shock in the absence of divalent cations was not effective (line D). From the results presented in lines F and G, it appears that Ca²⁺ should at least be present during the post-heat shock period. Finally, the results presented in lines A, B, H, and I show that the cells were better fit for DNA uptake when a cold shock was applied after the heat shock.

Effect of the competence regimen on membrane integrity. What could be the role of the temperature shock in the presence of divalent cations for the development of competence? One possibility is that such a condition affects the integrity of the inner membrane or outer membrane or both of E. coli. We tested the effect of our competence regimen on outer membrane.
membrane integrity by assaying the apparent activity of β-lactamase in suspensions of AM1283, a derivative of AM1095 carrying plasmid R1dr4-19 coding for a periplasmic β-lactamase. Degradation of ampicillin by samples of the shocked cell suspension was assayed as a measure for β-lactamase activity. As the permeability of the outer membrane is the rate-determining step in the β-lactamase assay of whole cells (16, 28), this assay can be used to measure changes in outer membrane permeability. However, if leakage of periplasmic enzymes, including β-lactamase occurs, this would obviously enhance the apparent β-lactamase activity. We checked for leakage of β-lactamase by assaying the β-lactamase activity in the supernatant after centrifugation of temperature-shocked cell suspensions. Table 2 shows the dramatic increase in β-lactamase activity in cell suspensions that were treated by a cold → heat → cold shock in the presence of divalent cations compared with cells that were not shocked or were shocked only from 0 to 42°C. Shortly after the temperature shock, the increase was mainly due to increased permeability of the outer membrane, but after 60 min of further incubation at 0°C, the total increase in apparent β-lactamase activity was due to enzyme that had leaked out of the periplasm.

Apparently, major damage occurred to the outer membrane. The apparent β-lactamase activity assayed 60 min after the cold-heat-cold shock amounted to 31% (ranging from 30 to 36% in different experiments) of the total β-lactamase activity (assayed in samples of the same suspension but after the cells were converted into spheroplasts). Leakage of cytoplasmic enzymes did not occur under these circumstances, as the β-galactosidase activity in the supernatants of the suspensions of isopropyl-β-D-thiogalactopyranoside-induced cells varied from 0.5 to 1% of the total activity (assayed in toluen-

### Table 1. Influence of various competence regimens on transformation frequency

| Temp treatment (°C) | DNA | Ca²⁺ | Mg²⁺ | Relative transformation frequency
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>(A) 0 → 42 → 0</td>
<td>Before</td>
<td>Before</td>
<td>Before</td>
<td>1.0</td>
</tr>
<tr>
<td>(B) 0 → 42 → 0</td>
<td>After</td>
<td>Before</td>
<td>Before</td>
<td>1.1</td>
</tr>
<tr>
<td>(C) 0 → 0 → 0</td>
<td>Before</td>
<td>Before</td>
<td>Before</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>(D) 0 → 42 → 0</td>
<td>Before</td>
<td>After</td>
<td>After</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>(E) 0 → 42 → 0</td>
<td>After</td>
<td>Before</td>
<td>After</td>
<td>1.0</td>
</tr>
<tr>
<td>(F) 0 → 42 → 0</td>
<td>After</td>
<td>After</td>
<td>Before</td>
<td>0.6</td>
</tr>
<tr>
<td>(G) 0 → 42 → 0</td>
<td>After</td>
<td>None</td>
<td>Before</td>
<td>0.001</td>
</tr>
<tr>
<td>(H) 0 → 42 → 37</td>
<td>Before</td>
<td>Before</td>
<td>Before</td>
<td>0.1</td>
</tr>
<tr>
<td>(I) 0 → 42 → 37</td>
<td>After</td>
<td>Before</td>
<td>Before</td>
<td>0.01</td>
</tr>
</tbody>
</table>

*Details of the basic transformation procedure (line A) are presented in the text.

The frequencies are based on at least two different experiments. The absolute frequency in (A) amounted to 4 × 10⁻⁵.

Cells kept at 0°C were incubated at 42°C for 6 min and then brought at 0°C or 37°C as indicated. After 60 min at the latter temperature, DNase was added, and the mixture was incubated for another 30-min period.

DNA, Ca²⁺, and Mg²⁺ were added either before the transfer to 42°C or 3 min after the transfer from 42°C to the lower temperature indicated in the text.

### Table 2. Influence of heat shock on apparent β-lactamase activity in suspensions of AM1283

<table>
<thead>
<tr>
<th>Treatment</th>
<th>t = 0 min</th>
<th>t = 60 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 min, 0°C → 6 min, 42°C → 60 min, 0°C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 min, 0°C → 6 min, 42°C → 60 min, 37°C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>16 min, 0°C → 60 min, 0°C</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Suspensions (in HEPES buffer) of AM1283 cells mixed with AM1095 cells were prepared as described in the text; 0.4 ml of suspension was mixed with 0.1 ml of CaCl₂ or MgCl₂ (final concentrations, 30 and 26 mM, respectively) and heat shocked as indicated.

Expressed in arbitrary units, as described in the text.

t = 0, Time of chilling after incubation at 42°C (or the comparable time if no heat shock was applied).
treated cells) irrespective of shock treatment. Leakage of \( \beta \)-lactamase depended on the presence of either \( \mathrm{Ca}^{2+} \) or \( \mathrm{Mg}^{2+} \) ions or a mixture of these (Table 3). In the absence of divalent cations, only minor leakage occurred. Leakage of periplasmic endonuclease was also observed (details not presented), which shows that the effect of the competence regimen is not restricted to \( \beta \)-lactamase.

Thus, there is a clear, although not perfect, parallel between the effect of the competence regimen on transformation frequency and on leakage of periplasmic enzymes.

**Processes after DNA uptake.** Figure 2 presents an initial experiment in which the increase of transformation frequency after a second heat shock was demonstrated. The increase observed after a second heat shock in the case in which DNase treatment had been applied, implies that processes occurring after DNA adsorption and uptake are stimulated. Figure 3 gives the results of an experiment in which the parameters of post-DNase incubation were examined in detail.

Transformation was performed as described in Table 1, line A. After DNase treatment, portions of the transformation mixture were diluted 1:10 into MOPS-buffered minimal medium supplemented with growth requirements or into the same medium additionally supplemented with 10 mM \( \mathrm{CaCl}_2 \) and 10 mM \( \mathrm{MgCl}_2 \). After dilution, the mixture was further incubated at 37°C. During the incubation at 37°C in the presence of \( \mathrm{Ca}^{2+} \) and \( \mathrm{Mg}^{2+} \) ions, the number of viable cells remained constant, but the number of transformants increased by a factor of 2 to 3, resulting in a rise in the transformation frequency. During the incubation at 37°C in the absence of \( \mathrm{Ca}^{2+} \) and \( \mathrm{Mg}^{2+} \) ions, the number of viable cells doubled, whereas the number of transformants remained constant, resulting in a decrease in transformation frequency. When one of the growth requirements (leucine) was omitted, we observed no increase of transformation frequency (data not shown). After a 120-min incubation of the transformation mixture in medium with \( \mathrm{Ca}^{2+} \) and \( \mathrm{Mg}^{2+} \), it made no difference whether or not the plating medium contained these ions.

The second heat shock and the postincubation period probably serve as a recovery stage, giving the transformed cells an optimal chance to survive.

**DISCUSSION**

The main conclusion of our study is that it is possible to render *E. coli* cells competent for DNA uptake in the absence of DNA by transferring them from 0 to 42°C, preferentially followed by a cold shock. This development of competence is dependent on the presence of \( \mathrm{Ca}^{2+} \) or \( \mathrm{Mg}^{2+} \) ions. DNA subsequently added to the competent cells is taken up into a DNase-

![FIG. 3. Effect of postincubation at high temperature on transformation frequency. A transformation mixture, composed as described in the text, was heat shocked, incubated at 0°C for 60 min, and then treated with DNase (30 min, 0°C). Portions of this mixture were diluted into MOPS-buffered minimal medium that was not supplemented or was supplemented with 10 mM \( \mathrm{CaCl}_2 \) and 10 mM \( \mathrm{MgCl}_2 \). Time zero is the time of dilution into MOPS-buffered medium. At times indicated, the number of Leu" transformants in samples of the diluted mixtures was assayed either on Tris-buffered selective plates containing 10 mM \( \mathrm{CaCl}_2 \) and 10 mM \( \mathrm{MgCl}_2 \) or on Tris-buffered plates without additional divalent cations. Transformation frequencies are expressed as Leu" transformants observed per viable cell. (●—●) Incubation with added \( \mathrm{Ca}^{2+} \) and \( \mathrm{Mg}^{2+} \); transformants were plated on medium with \( \mathrm{Ca}^{2+} \) and \( \mathrm{Mg}^{2+} \). (●—○) Incubation with \( \mathrm{Ca}^{2+} \) and \( \mathrm{Mg}^{2+} \); transformants were plated on medium without \( \mathrm{Ca}^{2+} \) and \( \mathrm{Mg}^{2+} \). (●—△) Incubation without added \( \mathrm{Ca}^{2+} \) and \( \mathrm{Mg}^{2+} \); transformants were plated on medium with \( \mathrm{Ca}^{2+} \) and \( \mathrm{Mg}^{2+} \). (●——△) Incubation without \( \mathrm{Ca}^{2+} \) and \( \mathrm{Mg}^{2+} \); transformants were plated on medium without \( \mathrm{Ca}^{2+} \) and \( \mathrm{Mg}^{2+} \).
resistant form during an extended period at 0°C. The presence of Ca\(^{2+}\) ions is essential during DNA uptake. This fits with the ideas about the role of Ca\(^{2+}\) ions in the transformation process (20–22, 24).

It should be mentioned that homogeneous dilution of DNA into a solution containing Ca\(^{2+}\) ions, e.g., addition of DNA to Ca\(^{2+}\)-heat-shocked cells, is difficult. We observed a tendency of DNA molecules to "clump" in the presence of Ca\(^{2+}\) and in the absence of cells. In our experiments, care was taken to ensure quick and thorough mixing of the transformation mixture. Only under these conditions can reproducible results be obtained.

Separation of a competence induction stage and a DNA uptake stage can be achieved by the addition of DNA after the heat shock. Such separation might be advantageous to study detailed questions about _E. coli_ transformation, e.g., the role of divalent cations in the induction of competence and in DNA adsorption and uptake (cf. the hypothesis put forward by Grinius [8]).

In experiments in which DNA is added before the heat shock, the separation of competence induction and DNA uptake is not complete. In transformation with plasmid DNA, uptake of DNA occurs during the heat shock (0 → 42°C) (11). Preliminary experiments in our transformation system showed that uptake of chromosomal DNA at a low concentration (<5 µg/ml), such as is typically used in plasmid transformation, could not be enhanced by incubation at 0°C after the heat shock. A similar small amount of DNA was taken up during the heat shock when DNA was added at the high concentration used in the experiment described here. In this case, however, most conversion of DNA into a DNase-resistant form occurred during incubation at 0°C after the heat shock (Table 1, lines A and H). During the induction of competence, cells are subjected to temperature changes and treated with divalent cations. Such treatments may cause changes in lipid conformation and influence the functional role of lipids in the biological membrane (6, 19, 26, 27). The combination of temperature changes and a high concentration of either Ca\(^{2+}\) or Mg\(^{2+}\) could lead to specific conformational changes in membranes, allowing DNA uptake. Whether other cations that are reported to be effective in transformation, such as Ba\(^{2+}\) (25), can replace Ca\(^{2+}\) or Mg\(^{2+}\) has not been tested in our experiments.

We observed leakage of periplasmic enzymes during competence induction, probably as a consequence of outer membrane damage. There is a remarkable parallel between competence induction and leakage of periplasmic enzymes. The results of β-lactamase assays showed that about 30% of the total β-lactamase activity was set free from the periplasm. This implies either that each cell is rendered partially leaky or that about 30% of the cells release all β-lactamase activity into the medium, whereas the rest still contain all β-lactamase activity. The parallelism between competence induction and leakage of periplasmic enzymes could be very indirect. If 30% of the cells release all β-lactamase activity, they may have been severely damaged by the competence regimen. The competent-cell fraction could include cells in which damage to the outer membrane is more subtle than in the cells that leak periplasmic enzymes. The competent-cell fraction is probably much smaller than 30%; from cotransformation data of two different plasmids (11) or plasmid DNA and chromosomal DNA (3), a competent fraction of about 1% could be inferred. We observed that periplasmic endonuclease also leaked from competent cells. As the results (not shown) of transformation of an endonuclease-deficient strain were similar to the results shown for the endonuclease-proficient strain, the presence or absence of endonuclease apparently does not seem to influence the transformation process. Although we favor the idea that outer membrane disruption is essential for induction of competence in _E. coli_, it is quite clear that the outer membrane is not the only barrier for uptake of chromosomal DNA. In experiments in which revertible _E. coli_ spheroplasts were used for transformation with chromosomal DNA, the transformation efficiencies were similar to the (relatively low) frequencies obtained with Ca\(^{2+}\)-treated cells (23). If the cells that efficiently take up DNA have suffered some membrane damage, it could be expected that they are more fragile than normal cells. We have shown that postincubation of the transformation mixture at 37°C in the presence of Ca\(^{2+}\) and Mg\(^{2+}\) ions enhances the transformation frequency. This strongly suggests, as stated by Kushner (12), that some cells that have taken up DNA are not yet ready for outgrowth on selective solid medium, especially when the medium does not contain Ca\(^{2+}\) and Mg\(^{2+}\) ions.

We propose that in our transformation system three stages can be distinguished: (i) induction of competence by a heat shock followed by a cold shock; during this stage, limited amounts of DNA can be taken up; (ii) DNA uptake into a DNase-resistant form which can start or proceed during further incubation at 0°C; (iii) processes after DNA uptake (tentatively considered recovery processes).

ACKNOWLEDGMENTS

This work was supported by the Netherlands Foundation for Chemical Research (SON) with financial aid from the
Netherlands Organization for the Advancement of Pure Research (ZWO).

E. J. J. Lugtenberg is acknowledged for advice about measurement of periplasmic leakage.

LITERATURE CITED


