Natural Plasmid Transformation in *Escherichia coli*

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**Key Words**
Transformation • Natural transformation, plasmid • *Escherichia coli* transformation • Transformation

**Abstract**

Although *Escherichia coli* does not have a natural transformation process, strains of *E. coli* can incorporate extracellular plasmids into cytoplasm 'naturally' at low frequencies. A standard method was developed in which stationary phase cells were concentrated, mixed with plasmids, and then plated on agar plates with nutrients which allowed cells to grow. Transformed cells could then be selected by harvesting cells and plating again on selective agar plates. Competence developed in the lag phase, but disappeared during exponential growth. As more plasmids were added to the cell suspension, the number of transformants increased, eventually reaching a plateau. Supercoiled monomeric or linear concatemeric DNA could transform cells, while linear monomeric DNA could not. Plasmid transformation was not related to conjugation and was recA-independent. Most of the *E. coli* strains surveyed had this process. All tested plasmids, except pACYC184, could transform *E. coli*. Insertion of a DNA fragment containing the ampicillin resistance gene into pACYC184 made the plasmid transformable. By inserting random 20-base-pair oligonucleotides into pACYC184 and selecting for transformable plasmids, a most frequent sequence was identified. This sequence resembled the bacterial interspersed medium repetitive sequence of *E. coli*, suggesting the existence of a recognition sequence. We conclude that plasmid natural transformation exists in *E. coli*.

Among three modes of gene transfer in eubacteria, conjugation and transduction can be considered to be the side effects of plasmid or bacteriophage transmission. In contrast, transformation might have evolved to incorporate DNA from the environment [14, 20, 21]. Many genes participate in this complex process which involves DNA release from donor cells, development of a competent state, recognition of a specific sequence, DNA uptake and DNA integration [20, 21]. The advantage of this process might be to exchange advantageous properties originating from separate individuals, to repair damaged DNA, or to utilize extracellular DNA as nutrients [13]. These hypotheses are not mutually incompatible, since one process of DNA uptake might serve several functions.

Plasmid is usually transmitted between bacteria by conjugation. Extracellular plasmid is in danger of being destroyed. It can get into the cell by exploiting its DNA uptake process. Since plasmid usually exists in the supercoiled form, its entry into the cell and survival inside may be difficult if it utilizes the same natural transformation mechanism.
process evolved for linear bacterial DNA. In gram-positive *Bacillus subtilis*, plasmid has to be multimeric to be transformable [6]. The transformation frequency is much lower than that of linear bacterial DNA. These properties may reflect both the difficulty of entry and the hostility that cells have evolved toward an invading foreign genetic entity.

Natural transformation has been found in 29 genera of bacteria [14]. In the extensively studied *E. coli*, natural transformation, even though first reported in 1946 by Boivin [3], has never been confirmed. In this report, we demonstrate that *E. coli* can incorporate plasmids without special treatment [12]. Factors influencing plasmid up-take and transformability were characterized.

### Materials and Methods

**Strains and Culture Conditions**

Strains and plasmids used are listed in table 1. *E. coli* strains were cultured in Luria-Bertani broth or M9 medium supplemented with Ca²⁺ (0.1 mM), Mg²⁺ (2 mM), and glucose (5 mM) at 37°C. When cells carried antibiotic-resistance markers, the growth medium was supplemented with ampicillin (100 μg/ml), kanamycin (50 μg/ml) or streptomycin (10 μg/ml).

**Plasmid and Chromosomal DNA Isolation**

Plasmid DNA extraction was carried out using the alkali lysis method of Birnboim and Doly [2] as described by Sambrook et al. [16]. Chromosomal DNA was prepared using the method of Silhavy et al. [19]. Restriction digestion of purified plasmids and agarose gel (0.8%) electrophoresis was done according to Sambrook et al. [16].

### Table 1. Strains and plasmids used

<table>
<thead>
<tr>
<th>Strain</th>
<th>Relevant phenotype or genotype</th>
<th>Source or reference</th>
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<tbody>
<tr>
<td>CGSC4401</td>
<td>K-12 wildtype, F⁺</td>
<td>Bachmann, Ref. 8</td>
</tr>
<tr>
<td>CGSC5073</td>
<td>K-12 wildtype, F⁺</td>
<td>Bachmann, Ref. 14</td>
</tr>
<tr>
<td>ATCC23849</td>
<td>B str-r</td>
<td>ATCC, Ref. 8</td>
</tr>
<tr>
<td>ATCC23851</td>
<td>B thr</td>
<td>ATCC, Ref. 8</td>
</tr>
<tr>
<td>CA231 recA⁺</td>
<td>K-12 HfrC ΔlacZ ΔlacY⁺ Sm⁺ B1⁻</td>
<td>J. Bechwith</td>
</tr>
<tr>
<td>CA231 recA⁻</td>
<td>K-12 HfrC ΔlacZ ΔlacY⁺ Sm⁺ B1⁻ recA⁻</td>
<td>This study</td>
</tr>
<tr>
<td>DH1</td>
<td>K-12 supE hsdR recA endA gyrA thi relA</td>
<td>Ref. 12</td>
</tr>
<tr>
<td>VGH</td>
<td></td>
<td>Veterans General</td>
</tr>
<tr>
<td>9761</td>
<td>Clinical isolate</td>
<td>Hospital, Taipei</td>
</tr>
<tr>
<td>9763</td>
<td>Clinical isolate</td>
<td></td>
</tr>
<tr>
<td>9764</td>
<td>Clinical isolate</td>
<td></td>
</tr>
<tr>
<td>pBR322</td>
<td>Ap⁺ and Tet⁰</td>
<td>Ref. 4</td>
</tr>
<tr>
<td>pUC18</td>
<td>Ap⁺</td>
<td>Ref. 7</td>
</tr>
<tr>
<td>p151</td>
<td>lacZ gene in pUC18</td>
<td>Microgenics</td>
</tr>
<tr>
<td>pACYC184</td>
<td>Cm⁺ and Tet⁰</td>
<td>Ref. 7</td>
</tr>
<tr>
<td>pACYC177</td>
<td>Ap⁺ and Kn⁺</td>
<td>Ref. 7</td>
</tr>
<tr>
<td>pACYC184-177A</td>
<td>Cm⁺, Ap⁺ and Tet⁰</td>
<td>This study</td>
</tr>
</tbody>
</table>

**Spot Test**

Initial detection of natural plasmid transformation in starved cells was done as follows. Stationary phase *E. coli* K12 cells (CGSC4401) were plated on M9 agar plates. Plasmid p151 (fig. 1b) (40 μg in 20 μl), ampicillin (10 mg/ml, 10 μl) and lactose (250 mM, 20 μl) were spotted on this surface in five combinations. After a 12-hour incubation at 37°C, tiny colonies on plates were replicated on LB-Ap plates to verify their antibiotic resistance.

**Transformation Induced by Ampicillin and Cell Lysate**

Cell lysate was prepared by passing stationary phase CGSC4401 cells through a French press and centrifuging the resultant lysate at 12,000 rpm for 30 min to sediment intact cells and debris. In the experiment described in figure 2, stationary *E. coli* K12 CGSC4401 cells were concentrated from 1 ml to 100 μl. To this cell suspension were added glucose (10 μl, 250 mM), plasmid p151 (30 μl, 5 μg/μl) and cell lysate (40 μl, equivalent to 10⁶ cells) or ampicillin (20 μl, 100 mg/ml). The mixture was then plated on M9 agar and incubated at 37°C. After every 2 h, DNase I (2 mg/ml, 40 μl) was added to the agar surface and thoroughly mixed with the cells. After another hour at 37°C, cells were replica-plated on LB-Ap (100 μg/ml) plates and incubated at 37°C for 16 h to score for transformants. Each point is the average of 5 plates.

**Competence Test in Lag Phase, Log Phase, and Stationary Phase Cells**

Stationary cells stored at 4°C had a lag phase of 9 h when put back onto fresh M9 glucose plates at 37°C. To test lag phase cells for competence, 2 × 10⁸ stationary phase cells were mixed with plasmid p151 (2 μg), then plated on M9 glucose plates and incubated at 37°C for 3 h. Plasmids were then digested away with the addition of DNase I. After 1 h at 37°C, cells on M9 glucose plates were collected using M9 medium and washed 3 times in M9 medium. Cells were plated on LB-Ap plates to score for ampicillin-resistant colonies. To test stationary phase cells for competence, cells and plasmids were plated on M9 plates without glucose. The rest of the treatment was the same as...
Fig. 1. Ampicillin and sugar induced starved cells to take up plasmid. a Natural transformation on plate. A = Ampicillin; P = plasmid p151; L = lactose. Only cells given lactose, ampicillin and plasmid DNA gave rise to transformants. This LB-Amp plate was replica-plated from the original M9 plate which contained starved cells. b Plasmid p151. It contains colEl replicon, β-lactamase gene (Ap'), β-galactosidase gene (lacZ) off the λ P_R promoter. The λ c1857 repressor controlled λ P_R.

Fig. 2. Transformation induced by lysate is 6 h earlier than by ampicillin. □ Cells treated with lysate; ■ cells treated with ampicillin.

for lag phase cells. To test log phase cells, stationary cells were first cultured in M9 glucose medium for 9 h, concentrated, mixed with plasmids, and then plated on M9 glucose plates. The rest of the treatment was the same as for lag phase cells.

Regular Plate Assay

After knowing that competence was higher in lag phase cells, an assay procedure was developed. It was the same as the treatment for lag phase cells, except plasmid was not digested using DNase I, but was washed away using M9 medium.

Transformation Using Different E. coli Strains or Different Plasmids

When testing different strains for transformation by p151, plasmid (1.2 mg/ml) was added to 10⁶ 3-day-starved cells. Then the regular plate assay was used. When testing pACYC184 plasmid for transformability, p151 (1.2 mg/ml), pACYC184 (12 mg/ml) and glucose (25 mM) were spread on M9 agar with 10⁹ 5-day-starved CGSC4401 cells. After 12 h at 37°C, cells were replica-plated on either LB-Amp or LB-chloramphenicol plates to score for transformants by either p151 or pACYC184.

Chemicals

Restriction endonucleases were purchased from New England Biolabs (Beverly, Mass., USA). Chemicals were purchased from Sigma Chemical Co. (St. Louis, Mo., USA). Yeast extract, tryptone and agar were purchased from Difco (Detroit, Mich., USA).

Results

Plasmids Can 'Naturally' Transform E. coli Cells

We explored the possibility of 'natural' plasmid transformation using a spot test. Stationary phase E. coli cells were spread on an M9 minimal salt agar surface without
calcium or magnesium ions. Plasmid, lactose or ampicillin solutions were then spotted on the surface to cover a small area of cells. After 12 h, cells were inoculated by replica plating onto LB-Ap agar to select for ampicillin-resistant cells. Drug-resistant colonies appeared only in the circle where plasmid, lactose and ampicillin were added. Cells from these colonies were found to contain the plasmid. Thus lactose and ampicillin together seemed to allow cells to incorporate extracellular plasmid. Plasmids contained in these colonies were then analyzed using restriction endonucleases. The number and size of restriction fragments of 10 isolates, digested by either EcoRI, BamHI or PstI, were exactly the same as those of the original plasmid (data not shown). We then investigated why ampicillin was needed to induce transformation in the spot test. Since ampicillin kills most growing cells, we postulated that lactose allowed some cells to grow and be killed by ampicillin. The released cell content then stimulated surviving cells to incorporate extracellular plasmid DNA. If this were true, then cells should be transformable when supplied with cell lysate in lieu of ampicillin. As shown in figure 2, cells given lysate, plasmid and glucose were indeed transformable. The initial appearance of transformants was earlier in the lysate-treated cells than in ampicillin-treated cells (2 vs. 8 h), implying that ampicillin-induced transformants take longer to appear than lysate-induced ones.

Although spot tests indicated that cell lysate or ampicillin was required for plasmid transformation, later detailed experiments revealed that neither was necessary. Lysate or ampicillin might have augmented the transformation frequency so as to allow detection of transformants in spot tests. Only glucose or lactose was needed. A regular plate assay (detailed in Materials and Methods) was used for subsequent tests.

**Competence Higher in Lag Phase Cells**

Since starved cells had to be supplied with glucose or lactose to be transformable, this indicated that starved cells may not be the most receptive ones. To find whether there is a ‘competence’ state, plasmids were added to cell cultures in the lag, log or stationary phase for 1 h. Transformants were then scored. Lag phase cells were found to be most ‘competent’. The transformation frequency was lower than $10^{-11}$/cell for log phase cells, $1.0 \times 10^{-8}$/cell for stationary phase cells, and $1.6 \times 10^{-5}$/cell for lag phase cells.

When more plasmids were mixed with cells, more transformants appeared, but eventually a plateau was reached (fig. 3). The linear relationship in the left part of figure 3 indicates that one single DNA molecule may transform one cell. When equal amounts of plasmid DNA were given to increasing numbers of cells, the transformation frequency decreased (fig. 4).
The effect of magnesium and calcium ion on plasmid transformation was studied. In standard plate assay, M9 buffer without added Ca\(^{2+}\) or Mg\(^{2+}\) allowed transformation to happen. When EDTA (2 mM) was present in the M9 (no Ca\(^{2+}\) and Mg\(^{2+}\) added) plate, it totally abolished plasmid transformation. Ca\(^{2+}\) at 0.1 mM allowed transformation, but higher concentrations of Ca\(^{2+}\) did not produce higher frequencies of transformation. Increasing concentrations of Mg\(^{2+}\) did produce higher frequencies of transformation, but the highest stimulation at 20 mM of Mg\(^{2+}\) was only 15- to 20-fold that of the control experiment in which no Mg\(^{2+}\) or Ca\(^{2+}\) was added to M9 buffer. We, therefore, supposed that the divalent cation Ca\(^{2+}\) or Mg\(^{2+}\) played only a permissive function for plasmid natural transformation to occur and Mg\(^{2+}\) had a better effect. The different effects of Ca\(^{2+}\) and Mg\(^{2+}\) indicated that this plasmid transformation was distinct from the artificial transformation of plasmid using high concentrations of calcium chloride.

Can transformation occur in liquid suspension? In ten attempts to get transformants by mixing cells and plasmids in liquid suspension, no transformant formed, indicating that E. coli cannot be naturally transformed by plasmid in liquid.

**The Effect of Plasmid Structure on Transformation**

In plasmid natural transformation, the structure of the plasmid affects the transformation activity. We prepared three forms of pBR322 plasmid DNA to test their transformability in E. coli. Supercoiled DNA was purified from agarose gel after electrophoresis. Linear DNA was prepared by digesting supercoiled DNA with restriction endonuclease EcoRI. The concatemeric form was prepared by ligating linear DNA using T4 DNA ligase. By using the regular plate assay, we found that supercoiled and concatemeric pBR322 could transform E. coli, while linear pBR322 could not.

Plasmids linearized separately with either one of two restriction endonucleases could still transform E. coli when they were mixed together for artificial transformation. In order to know whether similarly treated plasmid could transform naturally, pBR322 was digested with the following enzymes separately: Avai, EcoRI, BamHI, PvuI, BsaAl, Bsal, PstI, PvuI. Linearized plasmid was purified from agarose gel and all eight kinds were mixed together in equal amounts. The mixtures were then used to naturally transform E. coli cells. No transformant was ever obtained. In control experiments using the artificial transformation method (calcium chloride treatment and heat shock), transformants were obtained.

**Transformation Occurred in Most Tested E. coli Strains**

Previous experiments employed E. coli strain CGSC4401. Other strains were tested. All strains listed in table 1 except VGH9761 could be transformed by p151 and pBR322. A comparison of their genotypes indicated that fertility factor F, prophage \(\lambda\) and the recA gene may not be critically involved in plasmid transformation.

**Untransformable Plasmid Enters Cells when Assisted**

Many kinds of plasmids were tested for transformability (table 1). Plasmid pBR322 and its derivatives with inserted E. coli chromosomal DNA could transform E. coli. Plasmid pACYC184 did not transform CGSC4401 cells when given alone or together with p151 in a ratio of 10 (pACYC184) to 1. This amount of p151 generated more than 200 transformant colonies. A mixture of pACYC184 with pBR322 did yield natural transformants. Among the 293 ampicillin-resistant colonies, only 5 colonies (1.7%) contained both kinds of plasmid. No transformant containing pACYC184 alone was found. When CGSC4401 cells already harboring pBR322 were tested as likely recipients, pACYC184 still could not transform cells. Transformable plasmid seemed to aid untransformable plasmid to enter cells when both were outside. When the former was already inside, it could not help the latter.

**Some Sequences Helped Plasmid to Transform**

The inability of pACYC184 to transform cells implies that E. coli selectively admits plasmids into its cytoplasm. Since all plasmids with ampicillin resistance genes could transform, this gene (the 2,208-base pair DNA fragment generated by digesting pACYC177 with restriction enzymes BamHI and NheI) was cloned to pACYC184 (a 2,958-base pair DNA fragment generated by digesting pACYC184 with BamHI and NheI) to test whether any specific sequence allowed transformation. The resultant plasmid pACYC184-177A could naturally transform strains CGSC4401 and CA231. Since this fragment is long, a plasmid library was constructed by cloning random 20-base-pair-long oligonucleotides into pACYC184. This library was used to naturally transform CA231. One hundred and twenty colonies were picked and the inserted oligonucleotides sequenced. There were 62 kinds of sequences. Those occurring more than once are listed in table 2. One sequence GAAGGAAGATCTTCACCAGT occurred 15 times (12.5%) and was called the most frequent sequence (MFS). Although the MFS was not present in the E. coli genome, this MFS shares partial homology with the palindromic unit (PU) of the bacterial
Fig. 5. Partial homology between transformable sequences and motifs of BIME. IR = Inverted repeat.

### Table 2. Oligonucleotide sequences which made pACYC184 transformable

<table>
<thead>
<tr>
<th>Sequence</th>
<th>Times of appearance</th>
</tr>
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<tbody>
<tr>
<td>MFS</td>
<td>15/120</td>
</tr>
<tr>
<td>CTAGCCGGTTAATAGC</td>
<td>5/120</td>
</tr>
<tr>
<td>GGTGCCAGAGTCTTTGAC</td>
<td>5/120</td>
</tr>
<tr>
<td>CTCCGAGAGATAAGCA</td>
<td>3/120</td>
</tr>
<tr>
<td>TTTCCACCTAGACTCGTAC</td>
<td>3/120</td>
</tr>
<tr>
<td>TTTCCCACCTAGACTCGTA</td>
<td>3/120</td>
</tr>
<tr>
<td>TACCCGACGACTGTTGCA</td>
<td>2/120</td>
</tr>
<tr>
<td>AAGTATTGGAGAATAAGGG</td>
<td>2/120</td>
</tr>
<tr>
<td>TTTTCTGGATAGAAATGGG</td>
<td>2/120</td>
</tr>
<tr>
<td>ATAGATTTGCTTAAATCGT</td>
<td>2/120</td>
</tr>
<tr>
<td>FSI</td>
<td>2/120</td>
</tr>
</tbody>
</table>

Interspersed mosaic elements (BIME). Another sequence, FS 1, shared partial homology with the inverted-repeat region of the L1 and L2 motifs of the BIME (fig. 5).

**Discussion**

The salient features of this process can be summarized here. Lag phase cells are more receptive to extracellular plasmid than either stationary phase cells or exponential phase cells. Concatemeric linear, monomeric circular or supercoiled forms of plasmid can transform, whereas linear monomer cannot. The uptake of plasmid by the cell depends on the presence of a specific DNA sequence on the plasmid.

Can plasmid exploit chromosomal DNA transformation in order to infect a new host? Plasmid DNA may have to pass through the machinery evolved for taking up linear DNA. This poses difficulties for circular or supercoiled DNA since the uptake process involves binding, double-stranded DNA cutting, engulfing into transformosome, or single-stranded DNA digesting. Plasmid DNA may be fragmented during this process. This hypothesis explains why the frequency of plasmid transformation is much lower than that of chromosomal markers [5, 6, 9, 10, 15, 17, 18, 22]. The inability of monomeric plasmid to transform and the requirement for a homology region on the chromosome could also be explained.

_E. coli_ does not have a natural transformation process. The existence of plasmid natural transformation raises new questions. Can we hypothesize that DNA transformation originally existed but was replaced by conjugation and transduction and became vestigial? This might explain both the low frequency of transformation and the specific uptake of certain sequences.

Some _E. coli_ intergenic DNA sequences are composed of repeated BIME. About 500 BIMEs are scattered over the genome [1, 11]. These BIMEs are composed of a combination of different conserved motifs. One of these, the PU, was further subdivided into three variants, Y, Z1 and Z2, differing slightly in sequence. Other motifs include seven PU-flanking sequences, called S, L, s, 1, r, A and B.

One feature of the PU is the inverted repeats near both ends of the motif. Presumably it can form a stem and loop...
structure when the double strands of the PU are opened. The MFS which we identified as necessary for plasmid pACYC184 to transform is partly homologous with the inverted-repeat sequence of PU at 12 nucleotides. FS 1 is also partly homologous with motifs L1 and L2 (two varieties of L) at 8 nucleotides. These homologies suggest two possibilities. First, motifs PU and L in BIME may be the recognition sequence for E. coli DNA transformation just like the 11-nucleotide sequence AAGTGCGGTCA for Haemophilus influenzae. The MFS may mimic the recognition sequence so as to help untransformable plasmid to transform. Second, the MFS may mimic PU and L motifs in binding to the integration host factor, DNA gyrase or DNA polymerase 1. The binding might protect plasmid from digestion and eventually allow the survival of plasmid in the cytoplasm.

Although BIMEs are found in several enterobacteria, their functions are unknown. mRNA stabilization, transcription termination, translational control, and genomic rearrangement were proposed as possible functions. Since each BIME is composed of 2 or more motifs, it may have several functions simultaneously. The hypothesis that PU and L motifs are vestigial recognition sequences for natural transformation deserves attention.

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References