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Genetic Transformation in *Escherichia coli* K12

(DNA/$CaCl_2$/recombination/genetic linkage)

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ABSTRACT **An auxotrophic strain of *E. coli* K12 treated with $CaCl_2$ was transformed for several markers at a frequency of up to 10^{-6} per recipient cell by a DNA preparation isolated from a prototrophic strain. The transforming activity of the DNA preparation was eliminated by treatment with DNase, heat, or sonication, whereas RNase or Pronase treatment had little effect. Two closely linked genetic markers (*leu* and *ara*) showed a high degree of cotransformation linkage when high molecular weight DNA was used, but the linkage was almost completely eliminated when sheared, smaller molecular weight DNA was used. There is genetic evidence that the transformation is a result of the replacement of the preexisting genetic marker on the chromosome by that of the donor DNA.**

Since its discovery in Pneumococcus (1, 2), genetic transformation has been reported among several other bacterial species such as *Haemophilus influenzae* (3) and *Bacillus subtilis* (4). In *Escherichia coli*, especially in its K12 strain, however, attempts to demonstrate genetic transformation have been unsuccessful.

Several unique systems are known in *E. coli* in which the biological activity of isolated DNA can be assayed directly, such as transformations by bacteriophage λ DNA with helper phages (5). More recently, an efficient transfection system was developed by Mandel and Higa using $CaCl_2$-treated cells (6). In most examples of transfection and transformation, the DNA is either phage or plasmid (7) DNA, which is circular (or converts to circular forms in the cells).

During studies on the mechanism of ATP-dependent DNase (8–11) from *E. coli* in this laboratory, we have observed that the enzyme does not attack double-stranded circular DNA with or without nicks, whereas it causes extensive degradation of linear DNA molecules. Since fragments of bacterial chromosome are the source of genetic material in other general transformation systems, one of the reasons for the failure of genetic transformation in *E. coli* cells might be extensive degradation by the enzyme of linear DNA molecules lacking any protection mechanism. These characteristics of the enzyme plus reports of a mutant that lacks ATP-dependent DNase yet retains the capacity for genetic recombination (12) prompted us to examine the possibility of genetic transformation in *E. coli* K12. In this paper, we report the genetic transformation of *E. coli* K12 and some of its basic characteristics.

MATERIALS AND METHODS

Bacterial Strains used in this study are listed in Table 1. Genetic symbols are those used by Taylor (13).

Abbreviation: SDS, sodium dodecyl sulfate.

Reagents. RNase (pancreas, crystalline RNase A), DNase (pancreas, crystalline), and calf-thymus DNA were purchased from Worthington Biochemical Co. Pronase (grade B) was purchased from Calbiochem.

Isolation of Transforming DNA. Cells were grown in Pen assay broth (Difco), chilled, and harvested at the late exponential or early stationary phase by centrifugation. They were then suspended in one-fortieth of the original volume of cold 0.1 M NaCl–0.1 M EDTA (pH 8.5). 1 M Tris·HCl (pH 9.0)–10% SDS (sodium dodecyl sulfate) was added to give a final concentration of 0.1 M Tris·HCl and 1% SDS, followed by incubation at 42° for 2–5 min until the cell suspension became viscous. Immediately afterwards an equal volume of redistilled phenol (saturated with water) was added, and the samples were gently shaken for 20 min at room temperature. After centrifugation, the aqueous phase was carefully withdrawn and mixed with two volumes of 95% ethyl alcohol. Crude DNA was picked up with a glass rod while the samples were being mixed with alcohol. The DNA was then dissolved in the same volume of 0.1 strength of standard saline–citrate (15 mM NaCl–1.5 mM Na_3-citrate) that contained 5 mM EDTA. After the DNA was dissolved completely, one-tenth volume of 10-times the strength of standard saline–citrate (1.5 M NaCl–0.15 M Na_3-citrate) and 25 μg/ml of pancreatic RNase [treated in 0.1 M NaCl–10 mM acetate buffer (pH 5.2) by the method of Marmur (14)] were added, and the sample was incubated at 37° for 30 min. After incubation, 1 M Tris·HCl (pH 9.0) was added to give a final concentration of 0.1 M. After the phenol treatment was repeated, the aqueous phase was treated with ethyl ether (equal volume, 3 times) to extract the remaining phenol. The DNA was then precipitated with alcohol as described above and dissolved in an appropriate volume of 0.1 strength standard saline citrate (final DNA concentration, 100–400 μg/ml). For certain strains (MO611 and its derivatives), it was necessary that 200 μg/ml of Pronase (previously treated at 70° for 10 min) was present during the Tris–SDS treatment. Incubation at 42° was done for 60 min. The DNA thus prepared can be kept in a refrigerator with a drop of chloroform without measurable loss of biological activity for at least 8 months. As will be discussed later, biologically active DNA can be obtained reproducibly only when an endonuclease I-negative mutant strain (15) is used as the source of transforming DNA.

Transformation. The procedure was a modification of that originally discovered by Mandel and Higa for transfection of *E. coli* cells by bacteriophage λ DNA (6). All treatments

TABLE 1. *Bacterial strains used in this study*

E. coli K12 strain	Derived from	Relevant genetic characteristic used in this study*	Source
HfrC6		*end* (endonuclease I negative)	H. Hoffmann–Berling
AB1157(F^-)		*leu-6 ara-14 his-4*	B. Low
MO609(F^-)	AB1157	*end leu-6 ara-14 his*$^+$	This laboratory†
W3110			F. Imamoto
JC5519(F^-)	AB1157	*recB21 recC22 leu-6 ara-14 his-4*	A. J. Clark
JC7623(F^-)	AB1157	*recB21 recC22 sbcB15 leu-6 ara-14 his-4*	A. J. Clark
MO611(F^-)	JC7623	*end recB21 recC22 sbcB15 leu-6 ara-14 his-4*	This laboratory‡
MO617(F^-)	MO611	*end recB21 recC22 sbcB15 leu*$^+$ *ara-14 his-4*	This laboratory§
MO618(F^-)	MO611	*end recB21 recC22 sbcB15 lell*$^+$ *ara*$^+$ *his-4*	This laboratory§
MO626(F^-)	JC5519	*recB*$^+$ *recC*$^+$ *leu-6 ara-14 his-4*	This laboratory¶
MO627(F^-)	JC7623	*recB*$^+$ *recC*$^+$ *sbcB15 leu-6 ara-14 his-4*	This laboratory¶
MO619(F^-)	JC7623	*recB21 recC22 sbcB15 leu-6 ara*$^+$ *his*$^+$	This laboratory§

* Besides genetic characteristics listed here, the following markers are known: HfrC6 (*met*); W3110 (*trp*); AB1157, JC5519, JC7623, and their derivatives (*thr-1 thi-1 lacY-1 mtl-1 xyl-5 galK2 proA2 argE3 str-31 tex-33 sup-37 amber*). MO611, MO617, and MO618 require tryptophan (*trpBC-27*).

† Constructed by conjugation with HfrC6.

‡ *end* was introduced by a mutation with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine.

§ Constructed by transformation with HfrC6 DNA.

¶ Constructed by conjugation with HfrKL16(*leu*).

and manipulations were done at 0°–2°, unless specified. Three ml of overnight culture of the recipient cells grown in P medium (16) (supplemented with 100 μg/ml of required amino acids and 10 μg/ml of vitamin B1) were transferred to fresh medium (usually 80 ml) and incubated at 37° with rotatory shaking until the cell density reached 3×10^8/ml. The culture was chilled, centrifuged (6000 × *g*, 10 min), washed once with 40 ml of 10 mM $CaCl_2$, and suspended in the same volume of 25 mM $CaCl_2$. After having been kept at 0° for 20 min, the cells were centrifuged and suspended in 4–8 ml of 25 mM $CaCl_2$. 0.3 ml of the cell suspension was transferred to tubes that contained DNA (0.1 ml) and $CaCl_2$ (50 mM, 0.1 ml). The reaction mixture (0.5 ml) was incubated for 45 min at 0° followed by 10 min at 37°. The reaction was terminated by chilling the tubes at 0°, followed by addition of 1 μg of pancreatic DNase [50 μl of 20 μg/ml in 10 mM Tris·HCl–1 mM $CaCl_2$ (pH 7.6)]. 0.1 ml of samples (usually duplicate) was plated on selective plates (17) that contained minimal medium supplemented with required (nonselective) amino acids and vitamins. Transformants were counted after 36–48 hr of incubation at 37°. The same plates supplemented with all the requirements were used for counting of the total viable cells in the transformation mixture.

RESULTS

When the multi-auxotrophic *E. coli* K12 strain (JC7623), which lacks ATP-dependent DNase (*recB*$^-$ *recC*$^-$) and exonuclease I (*sbcB*$^-$) but retains recombination capacity, was treated with $CaCl_2$ and then exposed to DNA isolated from a prototrophic *E. coli* strain (HfrC6), a significant number of colonies arose on agar medium that would not support the growth of the original strain. For example (Table 2), after exposure to DNA, a small percentage of original strain that had required both leucine and histidine was now capable of growing without leucine in some cases and without histidine in others. $CaCl_2$ treatment of the recipient cells was essential for production of the colonies. These apparent transformants were stable for their newly acquired genetic characters and maintained them for many generations. The number of such apparent genetic transformants produced per test tube was roughly proportional to the amount of DNA added to it, up to 10 μg/0.5 ml. The maximum frequency of transformation under these conditions was about 10^{-6} for each genetic marker, which is considerably lower than that observed in the other bacterial transformation systems. Besides leucine (*leu-6*) and histidine (*his-4*) markers, the other available genetic markers, arginine (*argE3*), threonine (*thr-1*), and proline (*proA2*) requirements and ability to use arabinose (*ara-14*) as a sole carbon source, were also transformable. The transformation frequency for all these markers was similar to that observed for leucine and histidine. These results indicate that transformation events are not limited to a few particular genetic markers but occur generally for the available markers located at several places on the *E. coli* chromosome, thus suggesting that this system is similar to the other known bacterial transformation systems.

TABLE 2. *Transformation with various concentrations of DNA*

DNA (μg/0.5 ml)	No. of transformants Leu$^+$	His$^+$
0	1	2
1	53	28
2	109	38
4	128	62
6	184	68
10	354	126
10 (no cells)	0	0
10 (no $CaCl_2$ treatment)	3	10

Transformation was performed with HfrC6 DNA as donor DNA and JC7623 cells as recipients. Number of transformants is the sum of transformants produced on four plates with a total of 2.8×10^8 recipient cells. For cells without $CaCl_2$ treatment, the same cells were resuspended in P-medium instead of in $CaCl_2$ solution, and transformation was performed. Total recipient cells were 8×10^8.

TABLE 3. *Effect of various treatments of DNA on the transforming activity*

Treatment of DNA	No. of Leu+ transformants
(*A*) No treatment	335
DNase (1 μg/ml)	1
RNase (20 μg/ml)	349
Sonication	1
Denaturation (100°, 5 min)	4
Denaturation-renaturation	21
No DNA	2
(*B*) No treatment	280
Pronase (500 μg/ml)	411

(*A*) DNA (5 μg/0.5 ml) from HfrC6 and JC7623 cells were used as donor DNA and recipient cells, respectively. The number of Leu+ transformants is the sum of the transformants produced on four plates with a total of 2.8×10^8 recipient cells. All the treatments of DNA were done in 0.1 standard saline–citrate unless specified. Treatment with DNase was done at 37° for 20 min at DNA concentration of 50 μg/ml in 0.1 standard saline–citrate with 5 mM $CaCl_2$. Treatment with RNase was done at 37° for 20 min at DNA concentration of 50 μg/ml in 0.1 standard saline–citrate. For sonication, DNA solution (100 μg/ml) was treated by sonic oscillation (Raytheon, model DF 101) at maximum capacity for 5 min. For denaturation, DNA (100 μg/ml in 0.1 standard saline–citrate) was heated at 100° for 5 min, followed by quick chilling in ice-water. For renaturation, the heated denaturated DNA (100 μg/ml) was incubated at 69° for 4 hr in 2 × standard saline–citrate, followed by slow cooling to room temperature over a 10-hr period. After pulse heating (78°, 5 min), the DNA solution was dialyzed against 0.1 standard saline–citrate. (*B*) Same as (*A*), but total recipient cells were 2.1×10^8. Treatment with Pronase was done at 37° for 15 min at DNA concentrations of 268 μg/ml in standard saline–citrate, followed by one phenol treatment (see *Methods*).

We further investigated the nature of the active component in the DNA preparation. As shown in Table 3, prior treatment of the DNA preparation with pancreatic DNase completely eliminated the transforming activity, whereas treatment with RNase or Pronase had little effect. The activity was also lost after sonication or denaturation by heat treatment (100°, 5 min). In another experiment (not shown), when the DNA preparation was centrifuged in CsCl, the active transforming fraction coincided with the DNA fraction having the buoyant density of *E. coli* DNA (1.710 g/ml). These results indicate that the biologically active component in the DNA preparation is DNA, and that only double-stranded DNA is active for the transformation. We tested DNA preparations isolated from various sources. DNA from various *E. coli* K12 strains (Hfr as well as F−) could transform the auxotrophic recipients (Table 4). This was also the case when DNA from transformed cells was used as donor DNA for a second transformation. It should be noted, however, that we sometimes encountered difficulties in attempting to isolate active DNA from endonuclease I-positive cells, probably due to damage by the enzyme to the DNA during its isolation. DNA from *E. coli* B had essentially no transforming activity. This lack of activity may be due to restriction of *E. coli* B DNA in K12 cells.

TABLE 4. *Transformation with DNA from various sources*

Source of DNA (genotype)	No. of transformants Leu+	His+
No DNA	0	1
E. coli K12 HfrC6 (*leu*+ *his*+)	235	100
E. coli K12 F−MO609* (*leu*− *his*+)	0	75
E. coli W3110 (*leu*+ *his*+)	65	25
E. coli B	2	2
B. subtilis†	0	0
Calf thymus	0	1

Transformation was performed with 5 μg/0.5 ml of DNA and JC7623 as recipient cells. Number of transformants is the sum of the transformants produced on four plates with a total of 8×10^7 recipient cells.

* Derivative of AB1157 (see Table 1).

† Transforming DNA (168W DNA, gift from Dr. I. Smith).

TABLE 5. *Evidence for linkage by transformation*

Selected marker	Cotransformation with	No. cotransformants/no. examined (%) Untreated DNA	Sheared DNA
Leu+	Ara+	20/50 (40)	1/50 (2)
	His+	0/50 (0)	0/50 (0)
Ara+	Leu+	20/50 (40)	3/50 (6)
	His+	0/50 (0)	0/50 (0)
His+	Leu+	0/50 (0)	0/50 (0)
	Ara+	0/50 (0)	0/50 (0)

Transformation was performed with 2 μg/0.5 ml of HfrC6 DNA as donor DNA and JC7623 as recipient cells. Fifty randomly selected transformants were purified and examined for cotransformation.

We next compared the degree of the linkage of two very close genetic markers, using both high and low molecular weight DNA. The linkage between the leucine (*leu-6*) and arabinose (*ara-14*) markers was chosen for this purpose. The cotransduction frequency with bacteriophage P1 is close to 50% or more for various mutational markers in these two loci (13, 18). As shown in Table 5, when transformation was performed with high molecular weight DNA (untreated, estimated molecular weight 30 to 300 × 10^6), selecting separately for Leu+, Ara+, and His+ transformants, about 40% (20/50) of the leucine transformants were also Ara+, whereas none (0/50) of the Leu+ transformants was His+. This result was to be expected since the *his* marker is very distant from

TABLE 6. *Cotransformation of closely linked negative genetic markers*

DNA (genotype)	Selected marker	No. cotransformants/no. Leu+ transformants (%) Ara−	His−
MO617 (*leu*+ *ara*− *his*−)	Leu+	17/50 (34)	0/50 (0)
MO618 (*leu*+ *ara*+ *his*−)	Leu+	0/50 (0)	0/50 (0)

Transformation was performed with 6 μg/0.5 ml of DNA as donor DNA and MO619 (*leu*− *ara*+ *his*+) as recipient cells. Fifty randomly selected Leu+ transformants were purified and examined for cotransformation.

the *leu* and *ara* genes on the *E. coli* chromosome. Reciprocal tests confirmed the same degree of linkage between leucine and arabinose markers. The fact that the reciprocal linkages are identical is not significant, since the numbers tested are relatively small. The observed cotransformation frequency (40%) between leucine and arabinose with high molecular weight DNA seems to be a reasonable one relative to the linkage value found with P1 transduction, in which the transducing fragment has a molecular weight of 6×10^7 (19). In similar experiments with sheared, smaller molecular weight DNA (estimated average molecular weight, 8×10^6), only one of 50 leucine transformants was Ara$^+$ and three of 50 arabinose transformants were Leu$^+$. These results indicate that physical breakage of the DNA molecules causes loss of the genetic linkage originally observed, and further support the interpretation that the biologically active fraction in the DNA preparation must be DNA itself.

Another question to be answered in this transformation system was whether the donor DNA was integrated into the recipient chromosome. Although the observation that transformants are stable for their acquired characteristics suggested that integration occurred, we performed the following experiment: A recipient strain (*leu*$^-$ *ara*$^+$ *his*$^+$) was transformed with DNA derived from a strain possessing *leu*$^+$, *ara*$^-$, and *his*$^-$ markers. Leu$^+$ transformants were selected and tested to determine if they had incorporated the donor's *ara*$^-$ marker. As seen in Table 6, 34% of the Leu$^+$ transformants were Ara$^-$. In the control experiment (MO617 DNA) (Table 6), the absence of Leu$^+$ His$^-$ types demonstrated that the Leu$^+$ Ara$^-$ transformants were not due to double transformation events. The same control experiment with MO618 (*leu*$^+$ *ara*$^+$ *his*$^-$) DNA showed that the Leu$^+$ Ara$^-$ transformants are not the result of production of *ara*$^-$ mutations in or near recombination sites such as those reported by Yoshikawa (20). The transformation to a negative phenotype (Ara$^-$) makes it reasonable to conclude that the donor marker integrates into the homologous site of the recipient chromosome, replacing the preexisting marker.

The genetic basis for the transformability of the JC7623 (*recB21 recC22 sbcB15*) strain is not yet clear. Preliminary experiments with its parental strain, AB1157 (*recB*$^+$ *recC*$^+$ *sbcB*$^+$), as well as with two recombinants for these genes (MO626 and MO627) obtained by conjugation, has produced evidence for significantly less transformability. The strain that lacks a functional *rec* system (JC5519) is the only one that gave absolutely no evidence of transformability. These studies are continuing.

DISCUSSION

From our results it seems that transformation in *E. coli* K12 strains is similar to that of other bacterial transformation systems. There are, of course, other possibilities that could explain these results. For example, DNA of some thus far undetected phages or plasmids may be present widely in *E. coli* K12 strains and become associated with various chromosomal genetic segments. This DNA could be the source of the transforming activity in bulk DNA preparations. Alternatively, it is also conceivable that DNA of a particular structure, which represents only a small portion of the total DNA preparation, is responsible for the transformation. Such DNA could be derived from the *in vivo* intermediate of a cellular process such as DNA replication, recombination, or repair.

The reason for the low frequency of transformation and the requirement for high concentrations of DNA in this system is not clear. Perhaps *E. coli* cells inherently do not have an active uptake system for incorporating DNA so that a high concentration of DNA outside the cells is necessary. On the other hand, the failure to detect a double transformation of two unlinked markers (i.e., Leu$^+$ His$^+$) among transformants at even very high DNA concentrations seems to diminish the possibility that only certain highly transformable recipient cells are responsible for the transformation.

The possibilities of this transformation system in *E. coli* with its wealth and diversity of genetic and biochemical background material is for the most part self evident. Among the more intriguing possibilities is the potential for using this extremely well-characterized organism as a model to study the introduction and integration of genetic information from various sources.

NOTE ADDED IN PROOF

After submission of this manuscript we completed experiments that allowed us to conclude that the absence of both ATP-dependent DNase (*recB*$^-$ *recC*$^-$) and exonuclease I (sbcB$^-$) activity are major factors contributing to the transformability of the *E. coli* K12 strain (Oishi, M. & Cosloy, S. D., *Biochem. Biophys. Res. Commun.* in press).

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