GENETIC TRANSFORMATION IN E.COLI: THE INHIBITORY ROLE OF THE recBC DNASE

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SUMMARY

Genetic transformation of E.coli for various chromosomal markers was accomplished by (i) using recipient cells that lack the recBC DNase but were recombination proficient due to sbcA or sbcB mutations and (ii) treating the recipient cells with $CaCl_2$ at a concentration that facilitates transfection by λ DNA. Cotransformation of three markers (thr^+ ara^+ leu^+) was found to depend on the molecular weight of the transforming DNA.

INTRODUCTION

Genetic transformation has been found in several bacterial species (1) and has proved to be a valuable tool for various studies in molecular genetics. However, the most extensively examined bacterial species, E.coli, is generally considered to be non-transformable by its own chromosomal DNA, although there was a report (2) in which the authors claimed to have shown transformation with some E.coli strains. In a recent attempt Mandel and Higa (3) failed again to demonstrate transformation in E.coli, although they showed that under their conditions viable cells were able to take up complete phage DNA molecules (transfection).

We have reinvestigated the phenomenon of transformation in E.coli on the basis of observations on the transfection of E.coli spheroplasts by phage λ DNA (4), which can be summarized as follows: (i) Native DNA with double stranded ends loses its biological activity when it enters a spheroplast of E.coli $recB^+$ $recC^+$. (ii) The biological activity of such DNA is preserved in spheroplasts of strains which have mutations in the recB and/or recC genes leaving the cells deficient in recombination (5). (iii) Native linear DNA is also biologically active in spheroplasts prepared from strains which are recBrecC but have regained recombination proficiency by additional mutations, such as sbcA and sbcB strains (6, 7).

It has been shown that the $recB^+$ and $recC^+$ genes jointly code for an ATP-dependent DNase involved in genetic recombination (8, 9, 10). Therefore

we hypothesized that genetic transformation in E. coli might be observed under conditions where the transforming DNA inside a cell is not exposed to the destructive action of the recBC DNase and can be incorporated into the host chromosome by a recombination pathway independent of this enzyme.

MATERIALS AND METHODS

E. coli strains. The strains AB 1157 and AB2470recB21 (11) and JC7623recB21 recC22sbcB15 (7) all have the markers arg thi thr leu pro his strA; JC5183recB21 recC22sbcAlend (6); W3104strA. All the strains listed are F^-.

Media and solutions. Complete media (TBY) and minimal media (M9) were prepared as described earlier (12). EMB-arabinose agar contained 1% arabinose. Tris-HCl buffer of pH 7.5 was used at a concentration of 10^{-2}M and was referred to as Tris buffer.

Isolation of E. coli DNA. The cells of a growing culture of strain W3104 in TBY-broth (100 ml, about $3 \cdot 10^8$ cells/ml) were sedimented by centrifugation, washed with 50ml of 0.1M NaCl and then lysed by the procedure of Stonington and Pettijohn (13). The lysate (about 2.5ml) was immediately diluted five fold with Tris buffer containing 10^{-1}M NaCl and 10^{-3}M EDTA. An equal volume of redistilled phenol, equilibrated with the same buffer, was added and the mixture was gently shaken for 5 min. The sample was centrifuged for 10 min at 12000 x g and the aqueous phase was collected. At this stage a considerable amount of DNA was trapped in the interphase of denatured protein, but no effort was made to recover it. The preparation was extracted with phenol two more times and subsequently dialysed against Tris buffer containing 10^{-3}M EDTA. RNA was then degraded by pancreative RNase (50μg/ml) and T_1 RNase (10 units/ml) for 30 min at 37°. The RNases had been heated in aqueous solution at 97° for 10 min to destroy DNases. The DNA was again extracted by phenol and dialysed against Tris buffer containing 10^{-3}M EDTA.

Radioactively labeled DNA was prepared in the same way, except that the cells were grown for three generations in the presence of ^{32}P (1.5μC/ml) in low phosphate medium.

Transformation. The transformation assay was a modification of the transfection procedure developed by Mandel and Higa (1970). Recipient cells were grown in TBY-broth to a titer of about $2 \cdot 10^8$ cell/ml. The cells were treated with $CaCl_2$ (0.03M) and concentrated as described (3). The assay consisted of 0.1ml of precooled DNA solution in Tris buffer and 0.2ml of $CaCl_2$-treated cell suspension. The mixture was kept on ice for 15 min followed by 20 min of incubation at 37°. Then a sample of 0.1ml was plated usually on two plates with selective minimal medium. The total viable cell titer was determined on complete medium. When streptomycin resistance was used as a selective marker, 5ml of prewarmed TBY-broth was added to the transformation mixture after the incubation at 37° and the culture was aerated for 2h at 37°. The cells were collected by centrifugation and resuspended in 0.3ml of TBY-broth. Samples of 0.1ml were spread on TBY-agar plates containing streptomycin sulfate at a concentration of 40μg/ml. Colonies on plates were scored after two days of incubation at 37°. All transformation assays included controls for spontaneous revertants by substitution of the DNA by Tris buffer. The transformation experiments also included transfection assays (3) with the DNA of phage λc71 as a control for the $CaCl_2$-induced competence of the recipient cells. The efficiency of λ transfection is given as infective centers/λ DNA equivalent.

RESULTS

When cells of strain JC7623 (sbcB) were treated with $CaCl_2$ and exposed to sterile DNA isolated from W3104, subsequently, on selective medium, there appeared 10 to 100 fold more prototrophic clones for various markers than spontaneous revertants (Table 1). With sheared DNA the frequency of prototrophic clones increased 2 to 4 fold (Table 1). Similarly, when cells of strain JC5183 (sbcA) were treated with the sheared DNA preparation, the frequency of str^r clones increased between 6 and 20 fold above the spontaneous background ($5 \cdot 10^{-9}$).

	Transformants/4μg of DNA			
DNA	arg^+	thr^+	pro^+	his^+
unsheared	66	102	80	104
sheared	282	430	331	254
none	1	12	2	1

Table 1. Transformation of E.coli JC7623 for various genetic markers. The transformation assay (s. Materials and Methods) for each marker contained $5 \cdot 10^8$ cells which were spread on four parallel plates containing selective medium. The unsheared DNA of unknown molecular weight was forced at a concentration of 40μg/ml three times through a #22 hypodermic needle to obtain sheared DNA. The efficiency of λ transfection with the recipient cells was $4 \cdot 10^{-6}$.

In both strains no genetic changes were observed when the DNA was preincubated with 20μg/ml of pancreatic DNase for 10 min at 37°. When the cells were treated with 0.003M $CaCl_2$ instead of 0.03M $CaCl_2$, the transfection efficiency with λ DNA dropped by a factor of 10^3, probably because of inefficient uptake of DNA. Under these conditions no prototrophic clones were produced in JC7623 by the treatment with E.coli DNA. These results together with others described below satisfy the minimal criteria for genetic transformation.

To determine the effect of the molecular weight of transforming DNA on the frequency of transformation and possible cotransformation, we isolated ^{32}P labeled DNA from W3104. The molecular weight of the initial DNA preparation was reduced by two subsequent shear procedures. The sheared and unsheared

DNA preparations were used to transform cells of JC7623. $\underline{Thr}^+$ transformed cells were examined for the inheritance of two closely linked markers, $\underline{ara}^+$ and $\underline{leu}^+$. The results (Table 2) indicate, that DNA of a molecular weight of about $20 \cdot 10^6$

Molecular Weight of DNA (Range)	$\underline{thr}^+$ Transformants/ $2 \cdot 10^8$ Recipient Cells	% Cotransformation of Unselective Markers with $\underline{thr}^+$	
		$\underline{ara}^+$	$\underline{ara}^+\underline{leu}^+$
$62 \cdot 10^6$ ($130-30 \cdot 10^6$)	280	8 (8/100)	5 (5/100)
$23 \cdot 10^6$ ($30-16 \cdot 10^6$)	352	0 (0/200)	0 (0/200)
$9 \cdot 10^6$ ($16-4 \cdot 10^6$)	245	0 (0/100)	0 (0/100)

Table 2. Effect of the molecular weight (MW) of transforming DNA on the frequency of transformation and cotransformation. The MW of ^{32}P labeled transforming DNA was determined by neutral sucrose centrifugation using λ DNA as reference. The range of each MW was calculated by determining the MW at that point where the radioactivity equaled 0.5 that of the peak fraction, for both the left and the right shoulder of the peak. DNA from the initial preparation (MW $62 \cdot 10^6$) at a concentration of 100μg/ml was sheared by forcing it through hypodermic needles: three times through a #22 needle (MW $23 \cdot 10^6$) and subsequently five times through a #27 needle (MW $9 \cdot 10^6$). In each transformation assay (see Materials and Methods) 8μg of DNA were used. Cells of transformed $\underline{thr}^+$ clones were tested for the inheritance of the $\underline{ara}^+$ marker on EBM-arabinose agar and for the $\underline{leu}^+$ marker on selective medium. A transfection efficiency of $8 \cdot 10^{-6}$ was obtained with λ DNA and the recipient cells (JC7623).

is most effective for transformation. Cotransformation of $\underline{ara}^+$ and $\underline{leu}^+$ with the selective $\underline{thr}^+$ marker is abolished when the transforming DNA has a mean molecular weight of about $20 \cdot 10^6$. The cotransformation frequencies obtained with DNA of a molecular weight of $62 \cdot 10^6$ resemble the cotransduction frequencies found with phage P1 (14), which carries DNA of a molecular weight of $60 \cdot 10^6$ (15).

The transformation assay was linear within the range of DNA concentrations tested (Table 3), which indicates a one hit kinetics of transformation. In this respect transformation in E.coli does not differ from systems with other bacterial species (16).

µg DNA	*thr*$^+$-transformants/2·10^8 recipient cells
10	232
8	213
6	124
4	84
2	71
1	32
0	4

Table 3. Effect of various DNA concentrations on the transformation assay. Recipient cells of strain JC7623 were transformed (see Materials and Methods) with DNA of a molecular weight of 23·10^6. The efficiency of λ transfection with the cell preparation was 5.6·10^{-6}.

No transformation was obtained with *recB*$^+$*recC*$^+$ cells (AB1157) and with *recB* cells (AB2470). In the case of the first strain we would attribute this result to the presence of the *recBC* DNase in the cells and in case of the second strain transformation is probably abolished because of the inability of the cells to perform genetic recombination.

DISCUSSION

The two *E.coli* strains which we found to be transformable have in common, that they have lost the *recBC* DNase by mutations in its structural genes but were recombination proficient because of additional mutations, *sbcA* and *sbcB*, resp (6, 7). The activity of the *recBC* DNase on linear native DNA has been demonstrated *in vitro* (8, 9, 10). Its in vivo activity, apart from its (so far unknown) participation in recombination, was believed to be responsible for the destruction of the biological activity of linear transfecting phage DNAs, such as inverted λ DNA (4), T4 DNA (17) and T7 DNA (Wackernagel and Sriprakash, unpubl. results). Accordingly, the enzyme might also inhibit transformation by destroying the biological activity of transforming DNA, which has been shown, although indirect, in this paper.

A problem with the *E.coli* transformation system is still the low efficiency. Only about 10^{-6} cells could be transformed for a given marker in JC7623 at DNA saturation (data not shown). On the other hand the efficiency of transformation is about 10^{-6} cells per genome equivalent of DNA (molecular weight 3·10^9) for a given marker which corresponds favorably with the value for λ DNA which is

4 to $8 \cdot 10^{-6}$ infected cells per genome equivalent (see above). Since 10 to 100 fold higher transfection efficiencies for λ DNA have been obtained with E.coli spheroplasts (17, 18), the low transformation efficiency seems to be at least partially due to a limited DNA uptake by the $CaCl_2$-treated cells. The observation, that high molecular weight DNA has low transforming activity (Table 1 and 2) also argues for this interpretation. However, using the presented system it might be possible to improve further the efficiency of transformation. Even with the current level of efficiency it seems attractive to use this system to explore many interesting aspects of DNA metabolism in E.coli.

During the preparation of this manuscript a paper (19) appeared which, together with another report (20) also deals about genetic transformation in E.coli using a similar system as that described above. The results of these papers are in agreement with those presented above.

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