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Transformation in *Escherichia coli*: studies on the nature of donor DNA after uptake and integration

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SUMMARY

Chromosomal *E. coli* DNA appears to be sensitive towards *in vivo* DNA restriction when transformed to a restrictive *E. coli* recipient. It is therefore concluded that transforming chromosomal donor DNA is present in a double-stranded form immediately after uptake.

Genetic analysis of *E. coli* transformants, obtained with UV-irradiated donor DNA under conditions that exclude photorepair, show, especially in a *uvrB* recipient, loss of donor DNA information compared with the situation where DNA was not subjected to UV-irradiation. Similar conclusions were arrived at after genetic analysis of transductants obtained with UV-irradiated particles of the generalized transducing phage P1. The processing in *E. coli* of DNA after P1 transduction is thus similar to that of transforming DNA. The observations are discussed and a possible explanation based on single-stranded DNA integration is presented in detail.

1. INTRODUCTION

Transformation of *Escherichia coli* with linear chromosomal DNA can only be achieved efficiently in an exonuclease V (*recB* or *recC*) deficient strain, rendered recombination proficient by either a *sbcA* or *sbcB* mutation (Cosloy & Oishi, 1973; Wackernagel, 1973). Under optimal conditions, using temperature-shocked *E. coli* cells and with Ca^{2+} and Mg^{2+} present during the transformation, the process is still rather inefficient, yielding one transformant for a given single marker per 10^4 recipient cells (Reijnders *et al.* 1979). Ca^{2+}-treated and temperature-shocked 'competent' *E. coli* cells when used as recipients in conjugation or transduction are fully recombination proficient (Hoekstra & Zuidweg, unpublished results). The low yield of transformants is thus most likely the result of poor DNA uptake. Consequently, physical studies on the fate of transforming DNA after it has entered the cell are not yet feasible in *E. coli*. Despite the low efficiency of *E. coli* transformation, genetic studies are possible and have been performed. It turns out that relatively short pieces of DNA are integrated (Wackernagel, 1973) and based on genetic analysis of transformants it has been suggested that the donor DNA is integrated mainly as a continuous fragment, without additional crossing-over events (Hoekstra *et al.* 1976).

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Basic questions about *E. coli* transformation still are: (I) is donor DNA present in the recipient in single-stranded form or in double-stranded form, (II) if present in a double-stranded form is it integrated as such or are single strands of donor DNA integrated?

Whether transforming DNA is single stranded or double stranded immediately after uptake can be studied by testing its sensitivity towards restriction *in vivo*, as only double-stranded DNA in the non-modified form is susceptible to the *E. coli* K12/B restriction-modification system (Linn & Arber, 1968; Meselson & Yuan, 1968). Restriction of double-stranded *E. coli* donor DNA after conjugation or transduction causes a reduced yield of exconjugants or transductants and a reduction of linkage of unselected donor markers (Boyer, 1964; Pittard, 1964; Hoekstra & de Haan, 1965; Boyer, 1971). We will therefore compare the yield of transformants and linkage of unselected donor markers in transformation experiments with *E. coli* recipients carrying K12 or B host specificity and with *E. coli* K12 or *E. coli* B chromosomal donor DNA.

The question of single- or double-stranded integration will be approached less directly. When donor DNA is UV-irradiated before transformation the radiation damage can be restored in the recipient in several ways. If one transforms under conditions that do not allow photorepair or excision repair, post-replication repair is the only way to repair DNA damage. The main pathway of post-replication repair appears to be repair by recombination between the newly replicated daughter chromosomes (Rupp & Howard-Flanders, 1968). It is a reasonable assumption that a linear double-stranded donor DNA fragment will only be replicated after integration. Post-replication repair will thus affect integrated DNA. If donor DNA is integrated in a double-stranded form the information used for repair of UV damage in one donor strand could be derived from the complementary donor strand. If on the contrary donor DNA is integrated in a single-stranded form, a heteroduplex DNA structure will be formed. In that case the information used for post-replication repair of the UV-damaged donor strand will be derived from the newly replicated recipient strand or from other recipient chromosomes if the cells are multinucleate. Repair will then cause loss of donor DNA information. In this paper we present a genetic analysis of the fate of UV-irradiated donor DNA after transformation. The results suggest that single strands of donor DNA are integrated.

2. MATERIALS AND METHODS

(i) *Bacterial strains*

The *E. coli* strains used in this investigation are listed in Table 1. Chlorate-resistant mutants were induced by EMS and selected on chlorate medium under anaerobic conditions. Amongst the chlorate-resistant mutants a *chlC* mutant was isolated by testing gas production on L-broth (Guest, 1969). Genetic map positions of relevant chromosomal markers are presented in Fig. 1 (*a*).

Table 1. *List of strains*

Strain number	Relevant properties	Source or reference
PC 0031	Prototrophic *E. coli* K12	—
PC 0039	Prototrophic *E. coli* B	—
AM 1095	*car pdxA ara leu recB21 recC22 sbcB*	Hoekstra *et al.* (1976)
AM 1160	As AM 1095 but *uvrB*	From AM 1095 *gal*$^-$ × P1 *gal*$^+$ *uvrB*
AM 1218	As AM 1095 but $r_B^+ m_B^+$	From AM 1095 *thr*$^-$ × P1 960* (thr$^+$ $r_B^+ m_B^+$)
AM 1210	Harbours a Tc-insertion	Derivative of PC 0031 (Hoekstra *et al.* 1973).
AM 1232	*chlC*	*chlC* derivative of PC 0031, obtained after EMS treatment
AM 1252	*tonB-trp* deletion, *pyrF*$^-$	From strain AM 1160 *tonB-trp* deletion by preselecting T1 resistant derivatives
AM 1260	*trp pyrF*	From strain AM 1095

* Strain 960 is from Dr W. Arber's collection and was kindly provided by Dr T. Bickle (Basel).

(ii) *DNA isolation*

DNA was isolated as described by Cosloy & Oishi (1973).

(iii) *Transformation*

Cells were grown in minimal salts medium overnight at 37 °C. The culture was diluted 1 in 20 with fresh pre-warmed minimal medium and grown to an OD_{660} of 0·30. The cells were harvested by centrifugation and washed once in 0·25 vol. of 10 mM-NaCl at 0 °C. The cells were resuspended in 0·05 vol. of 20 mM HEPES (*N*-2′-hydroxyethylpiperazine-2-ethanesulphonic acid; Serva, Heidelberg), buffer pH 6·0. The recipient cells (0·3 ml) were mixed with 0·1 ml DNA (final concentration 10–40 μg/ml) and $CaCl_2$ and $MgCl_2$ were added to a final concentration of 30 and 26 mM respectively. The transformation mixture (0·5 ml) was chilled to 0 °C and then subjected to a heat pulse at 42 °C for 6 min. After chilling the tubes at 0 °C for 60 min, aliquots of 0·1 ml of appropriate dilutions were plated on selective plates containing 10^{-4} M-KH_2PO_4, 10^{-2} M-$CaCl_2$ and 10^{-2} M-$MgCl_2$. Dilutions were made in a 30 mM-$CaCl_2$ + 26 mM-$MgCl_2$ solution. This method, a modification of the Cosloy & Oishi (1973) procedure, allows a transformation efficiency of 10^{-4} (expressed as number of transformants for a given auxotrophic marker per viable recipient cell).

(iv) *Transduction*

Transduction was performed with the transducing phage P1 as described by Willetts, Clark & Low (1969).

(v) *UV-irradiation*

DNA solutions or P1 suspensions in buffer were irradiated with a UV source (Hanau Fluotest Piccolo 254); dose applied was measured with a J225 short wave

UV meter (San Gabriel, Calif.). Photoreactivation was excluded by working under dimlight conditions.

3. RESULTS

(i) *Influence of* in vivo *restriction on transforming DNA:*

To test the sensitivity of transforming DNA towards restriction *in vivo* the *E. coli* K12 strain AM1095 (see Table 1 details about the strains) and the derivative strain AM1218 that carries the *E. coli* B host specificity genes were transformed respectively with DNA isolated from a prototrophic *E. coli* K12 strain or with DNA isolated from a prototrophic *E. coli* B strain. Leu$^+$ transformants were

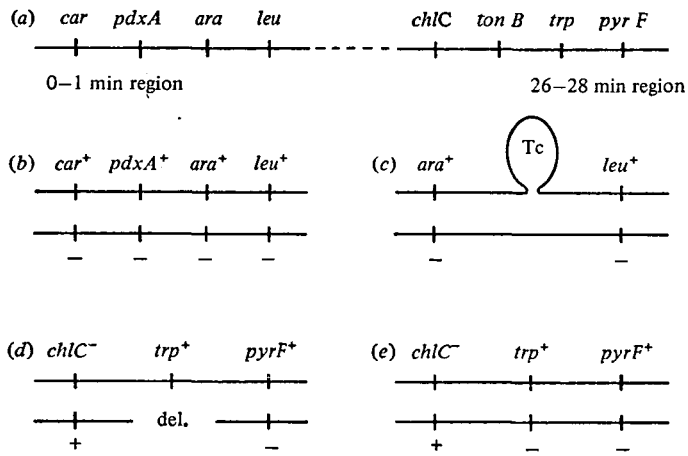

Fig. 1. (*a*) genetic location of relevant *E. coli* markers (*b–e*) outline of several genetic crosses.

Table 2. *Frequency of Leu$^+$ transformants with permissive or restricting host*

Recipient	Host spec.	e.o.p. λ K*	Frequency of Leu$^+$ transformants with *E. coli* K12 DNA	Frequency of Leu$^+$ transformants with *E. coli* B DNA
AM 1095	K12	1	1	0·14
AM 1218	B	0·07	0·06	1

* e.o.p. of phage λ was determined with Ca/Mg-treated, heat-shocked cells.

selected and analysed for co-inheritance of the donor markers *ara*$^+$ and *pdx*$^+$ (see Fig. 1 (*a*) for chromosomal location of genetic markers). The yield of Leu$^+$ transformants was 7–15 times lower with the restricting host than with the permissive host as recipient (Table 2). Genetic analysis of the Leu$^+$ transformants shows moreover that the linkage of unselected donor markers is always significantly reduced in the restricting host (Table 3). These observations are in accordance with results obtained after conjugation and transduction to a restricting *E. coli* host and

Table 3. *Genetic linkage of* leu*-linked markers in permissive and restrictive hosts after transformation and subsequent selection for Leu⁺ transformants*

Transformation	*ara*⁺/*leu*⁺	*pdx*⁺/*leu*⁺
K12 DNA × AM 1095	64/200 (0·32)	20/200 (0·10)
K12 DNA × AM 1218	23/186 (0·12)	7/186 (0·04)
B DNA × AM 1095	27/400 (0·07)	6/400 (0·02)
B DNA × AM 1218	154/400 (0·38)	64/400 (0·16)

strongly suggest that the transforming DNA is sensitive to restriction. Thus at some time after uptake transforming DNA is present as double-stranded non-modified DNA. The question of whether donor DNA is integrated as double strands or as single strands is therefore relevant.

(ii) *Effect of UV-irradiation of donor DNA on transformation*

UV-irradiation of donor DNA reduces the transforming ability (Fig. 2). As expected, this reduction is greater in a host that is deficient in excision repair, e.g. in a *uvrB* host. The results show that there is, within the range of the UV doses applied, an exponential inactivation of transforming ability.

A genetic analysis of Leu⁺ transformants obtained after transformation of *uvrB*⁺ and *uvrB* hosts with UV-damaged DNA is presented in Table 4. The most striking effect of irradiating donor DNA is that a decrease in genetic linkage is accompanied by the appearance of transformants in genetic classes that require multiple crossing-over events during integration (classes e–h in Table 4). Normally such transformants occur very infrequently (Hoekstra *et al.* 1976). If donor DNA were

Table 4. *Genetic analysis of Leu⁺-transformants isolated from strain AM 1095* (uvrB⁺) *or AM 1160* (uvrB) (*Fig. 1b*)

Unselected marker			Recombinant class	AM 1095: UV dose applied to DNA before transformation		AM 1160: UV dose applied	
ara	*pdx*	*car*		0 J/m²	270 J/m²	0 J/m²	180 J/m²
+	+	+	a	13	3	24	0
+	+	−	b	25	15	39	1
+	−	−	c	49	56	65	36
−	−	−	d	109	166	122	210
−	+	+	e	0	4	0	0
−	−	+	f	1	1	0	2
−	+	−	g	0	3	0	10
+	−	+	h	0	2	0	0
				197	250	250	250
Genetic linkage							
ara⁺/*leu*⁺				87/197 (0·44)	76/250 (0·30)	128/250 (0·51)	37/250 (0·15)
pdx⁺/*leu*⁺				38/197 (0·19)	25/250 (0·10)	63/250 (0·25)	11/250 (0·04)
car⁺/*leu*⁺				14/197 (0·07)	10/250 (0·04)	24/250 (0·10)	2/250 (0·01)

integrated in single-stranded form a heteroduplex structure would be formed and recombination repair would lead to exchange between donor and recipient genetic information. Transformants would therefore appear in recombinant classes formed by additional DNA exchanges. Especially in a *uvrB* host where repair by recombination is the only remaining pathway of radiation repair, such genetic exchanges might be expected to occur frequently. The experimental results show that, indeed, the effects in a *uvrB* host are more extensive than in the *uvrB*$^+$ host (Table 4). The most simple explanation for the results is to assume that single-stranded donor DNA integration occurs. Pursuing this line of reasoning we predict

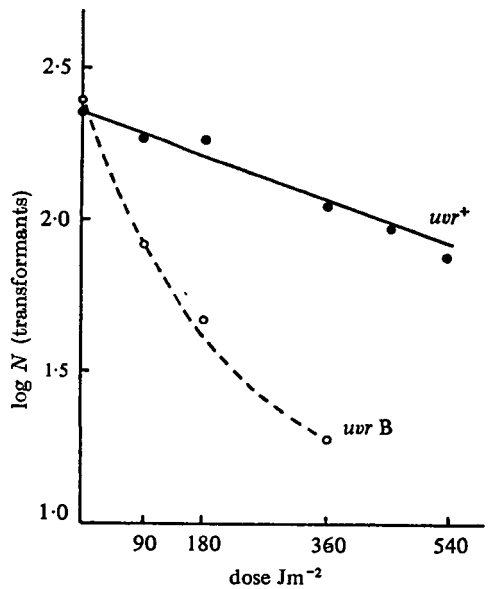

Fig. 2. Effect of irradiating donor DNA before transformation on the yield of transformants obtained under conditions that exclude photorepair. ●—●, Number of Leu$^+$ transformants obtained with strain AM1095 (*uvr*$^+$). ○---○, Number of Leu$^+$ transformants obtained with strain AM1160 (*uvr*B).

that a donor DNA region without a homologue in a *uvrB* recipient cell would be more sensitive to UV damage than other donor DNA regions, since the recipient cell cannot provide the complementary genetic information needed for post-replication repair.

A situation where the recipient contains no counterpart for the donor DNA could be created by using donor DNA with an insertion or by using a recipient with a deletion. We did experiments where we used donor DNA isolated from a prototrophic *E. coli* K12 strain carrying a Tc-insertion between the chromosomal markers *ara* and *leu* (Fig. 1*c*). The inserted DNA-fragment indeed appeared very sensitive to UV damage (Table 5A). After UV-irradiating the donor DNA the unselected Tc-determinant is not inherited amongst 350 selected Ara$^+$-transformants, while the more distal *leu*$^+$-donor marker is still found in nine cases. In

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Table 5(A). *Genetic analysis of Ara+ transformants obtained after transforming AM 1160 with DNA from AM 1210 (see Fig. 1c).*

UV dose applied to donor DNA before the transformation (J/m²)	Tc/*ara*+	*leu*/*ara*+
0	144/249 (0·58)	127/249 (0·51)
180	0/350 (< 0·003)	9/350 (0·02)

Table 5(B). *Genetic analysis of Pyr+ transformants obtained after transforming AM 1252 with DNA from AM 1232 (see Fig. 1d)*

UV dose applied to donor DNA before the transformation (J/m²)	*trp*+/*pyr*F+	*chl*C−/*pyr*F+
0	67/448 (0·15)	6/448 (0·01)
90	0/416 (< 0·002)	3/416 (0·007)

the control experiment using donor DNA that was not irradiated the unselected Tc-determinant is inherited by 57·8 %, the more distal *leu*+ donor marker by 51·0 % of the selected Ara+-transformants. In a similar experiment in which we used a recipient with a deletion (in the *trp* region, Fig. 1*d*) we again found high UV-sensitivity of the donor markers not present in the recipient (i.e. the *trp* region, Table 5B).

Table 6. *Genetic analysis of Leu+ transductants isolated from a transductional cross with P1.PC 0031 as donor and AM 1160* (uvrB) *as recipient (Fig. 1b)*

Unselected marker			Recombinant class	UV dose (J/m²) applied to P1 before transduction	
				0	180
ara	*pdx*	*car*			
+	+	+	a	143	2
+	+	−	b	125	24
+	−	−	c	104	98
−	−	−	d	119	321
−	+	+	e	0	5
−	−	+	f	3	18
−	+	−	g	3	22
+	−	+	h	3	10
				500	500
Genetic linkage					
ara+/*leu*+			375/500 (0·75)		134/500 (0·27)
pdx+/*leu*+			271/500 (0·54)		53/500 (0·11)
car+/*leu*+			149/500 (0·30)		35/500 (0·07)

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(iii) *Genetical effects of irradiation of transducing P1 particles on transduction*

The genetic effects observed in transformation after irradiating donor DNA was compared with effects on transduction after UV-irradiating the donor P1 particles. P1 particles were irradiated with a UV dose sufficient for at least a tenfold reduction of transduction frequency and then used to transduce *uvrB* recipients. Genetic analysis of the transductants showed effects similar to those found in the transformation studies (Table 6). UV-irradiation caused a decrease in genetic linkage of unselected markers and an increase in the frequency of transductants in the recombinant classes requiring additional crossing-over events. In a transduction experiment where we used a recipient with a chromosomal deletion (Fig. 1*d*), we again

Table 7. *Genetic analysis of Pyr$^+$ transductants isolated from a transductional cross: (a) P1.AM 1232 × AM 1252 (Fig. 1d), (b) P1.AM 1232 × AM 1260 (Fig. 1e).*

UV dose applied to P1 prior to transduction (J/m^2)	Cross	*trp*$^+$/*pyr*F$^+$	*chlC*/*pyr*F$^+$
0	a	220/300 (0·71)	89/300 (0·30)
120	a	5/350 (0·01)	20/350 (0·06)
0	b	198/250 (0·79)	66/250 (0·26)
180	b	89/250 (0·36)	12/250 (0·05)

found high UV-sensitivity of the donor DNA region for which a proper counterpart is lacking in the recipient (Table 7, cross a). A control experiment (Fig. 1*e*), in which the recipient strain carries a point mutation in the *trp* region instead of a deletion, shows that the *trp* region in itself is not extremely sensitive towards UV-irradiation (Table 7, cross b). A possible explanation for the transduction results in *E. coli* after UV-irradiating the P1 particles is therefore to conclude that there is single-stranded integration of the donor DNA, as proposed for the *E. coli* transformation.

4. DISCUSSION

(i) *The nature of transforming DNA after uptake by the cell*

Based on the *in vitro* properties of *E. coli* K12 and B restriction nucleases (Meselson & Yuan, 1968; Linn & Arber, 1968), we expect that restriction *in vivo* will only affect double-stranded, non-modified DNA. If transforming DNA is present in the cell in a double-stranded form the effect of *in vivo* restriction on transformation is expected to be similar to that observed in transduction or conjugation, i.e. reduced yield of transformants and reduction of genetic linkage of unselected markers. Such effects are indeed found whenever non-modified DNA is introduced into a restricting host by transformation (Tables 2, 3). Our results imply that at an early stage after uptake donor DNA is double-stranded and not yet modified. We therefore favour as a most simple model that DNA is taken up in

a double-stranded form. An alternative model could be uptake of single strands of donor DNA and subsequent conversion to double-stranded DNA, as in conjugation (Vapnek & Rupp, 1970). However, this would require synthesis of a complementary strand using random single-strand fragments without known origins of replication as templates.

(ii) *The processing of transforming and transducing DNA*

Our recombinant analyses show that the processing of transforming and transducing DNA is similar (Tables 4, 6). Transducing DNA is injected in double-stranded form, while for transforming DNA we favour double-stranded uptake. For simplicity we will assume in this discussion that after transformation and transduction native double-stranded donor DNA is present in the recipient. In experiments with UV-irradiated DNA or UV-irradiated P1 particles, donor DNA with UV damage enters the cell. The UV-damaged donor DNA fragment should not induce SOS repair, since its induction requires a replicon (Rosner, Kass & Yarmolinsky, 1968). One might postulate that UV-damaged donor DNA stimulates recombination *per se* in that fragments with damage are excluded during integration. Our experimental results could be explained by such a hypothesis. However, for *B. subtilis* it has been shown that UV-damaged donor DNA is efficiently integrated (Bron & Venema, 1972). If this also occurs in *E. coli* then recombinant DNA with UV-lesions will result. Replication of this recombinant UV-damaged DNA will result in daughter strands with gaps which must be repaired by genetic exchanges (Rupp & Howard-Flanders, 1968). Under conditions that exclude photorepair and excision repair post-replication repair is the only way for the cell to eliminate UV-lesions. If single strands of damaged donor DNA are integrated, damage in the donor strand can only be repaired by information obtained from recipient DNA. Repair would thus lead to loss of donor DNA information and the frequent occurrence of recombinants with alternating donor and recipient markers. If double strands of donor DNA were integrated one might expect that part of the damage in one donor strand would be repaired by information obtained from the other donor strand. However, damage repair using information obtained from other recipient chromosomes, provided that the cells are multinucleate, cannot be excluded. In the latter case, again, loss of donor DNA information and occurrence of multiple cross-over recombinants as observed in our experiments (Tables 4, 6) is predicted. The extremely low recovery of damaged donor DNA without a homologue in the recipient chromosome (Tables 5, 7) favours the single-strand integration model. In the double-strand integration model there is in general no reason to predict low recovery of such DNA regions. It could be that the integrated Tc-region involved in some experiments is more UV-sensitive than the flanking DNA regions. However, we could show (Table 7, cross b) that there is no intrinsic high UV-sensitivity for the *trp* region. The *trp* region only shows high UV-sensitivity under conditions when it is without a proper recipient homologue. Our suggestion that *E. coli* DNA after transformation is integrated in single strands is in line with observed single-stranded integration

after transformation in the pneumococcal, *H. influenzae* and *B. subtilis* system (Fox & Allen, 1964; Notani & Goodgal, 1966; Gurney & Fox, 1968). That after transduction with P1 donor DNA would be integrated as single strands is not in accordance with the results of Ebel-Tsipis, Botstein & Fox (1972), who showed for P22 transduction in *Salmonella typhimurium* that at least a substantial part of the donor DNA was integrated in the double-stranded form.

Finally, it should be stressed that our postulate of single-strand integration in *E. coli* transformation and generalized transduction is based on indirect genetic evidence and needs a physico-chemical proof.

(iii) *The nature of the recipient cell*

In the experiments described in this paper we used *recB recC sbcB* strains as recipients. In a recent paper (Mahajan & Datta, 1979) it was postulated that after conjugation with such recipients, where the recF pathway is open, mainly single-strand exchanges between donor and recipient DNA occur contrary to results with $recB^+$ $recC^+$ recipients. Since transformation in a $recB^+$ $recC^+$ recipient occurs with a rather low efficiency, it is hard to test the fate of transforming DNA during recombination in such recipients. However, in transformation and transduction studies with $recB^+$ $recC^+$ recipients, we found essentially similar results as in *recB recC sbcB* recipients (results not shown). Again, transduction with UV-irradiated P1 particles and transformation with UV-damaged DNA causes additional genetic exchanges and irradiated DNA segments without a counterpart in the recipient appear very vulnerable. Therefore the mechanism of integration does not seem different in a $recB^+$ $recC^+$ than in a *recB recC sbcB* recipient.

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