Genet. Res., Camb. (1972), **19**, *pp.* 181–186
Printed in Great Britain

The integration of autonomous transmissible plasmids into the chromosome of *Escherichia coli* K 12

By ERIC E. M. MOODY* and RICHARD RUNGE†

MRC Molecular Genetics Unit, Department of Molecular Biology, University of Edinburgh, Mayfield Road, Edinburgh EH9 3JR

(*Received* 7 *December* 1971)

SUMMARY

The highly selective technique of integrative suppression has been used to determine the ability of autonomous transmissible plasmids to integrate into the *E. coli* chromosome.

All the *F*-like transmissible plasmids appear to be able to integrate, suppress the initiation defect and form *Hfr*-type donor strains. However, there is no evidence of integration with the *I*-like transmissible plasmids examined.

1. INTRODUCTION

Autonomous transmissible plasmids or sex-factors are able to mediate the transfer of donor chromosomal genes to F^- recipient bacteria during conjugation (Lederberg, Cavalli & Lederberg, 1952; Hayes, 1953*b*; Clowes, 1961; Ozeki & Howarth, 1961; Clowes & Moody, 1966; Kahn & Helinski, 1964, 1965; Macfarren & Clowes, 1967; Meynell & Datta, 1965, 1966*a*, *b*; Sugino & Hirota, 1962; Moody & Hayes, 1972).

The isolation of *Hfr* donors from F^+ and $ColV_2^+$ strains of *Escherichia coli*, combined with the evidence supporting the Campbell (1962) model for the interaction of the *F* factor with the chromosome (Scaife & Gross, 1963; Broda, Beckwith & Scaife, 1964; Scaife & Pekhov, 1964) gives rise to the assumption that all transmissible plasmids which are able to mobilize the chromosome for transfer do so by inserting themselves into it. However, certain sex-factors can apparently transfer chromosomal genes by a mechanism which is different to that of the *F* sex-factor (Moody & Hayes, 1972). Moreover, the transmissible plasmid *ColIb* does not appear to possess an ability to integrate into the chromosome (Edwards & Meynell, 1969).

Nishimura, Caro, Berg & Hirota (1971) reported that when thermosensitive DNA mutants of *E. coli*, specifically defective in the initiation of chromosome replication and carrying a cytoplasmic *F* factor, were plated at the restrictive temperature (42 °C), the great majority (98 %) of 'revertant' colonies consisted of bacteria which retained the original *ts* mutation but were *Hfr*; the sex-factor had become integrated at various sites on the chromosome. This phenomenon was called *integrative suppression* and suggested that the defective bacterial initiation mechanism can be substituted by the intact mechanism of an integrated plasmid. However, when *Hfr* strains were isolated at the permissive temperature (30 °C), without recourse to selection for temperature-resistance, only a minority showed suppression of the initiation mutation. Since some other plasmids

* Department of Microbiology, College of Medicine, University of Arizona, Tucson, Arizona 85724.

† Department of Biology, Lake Forest College, Lake Forest, Illinois 60045.

Table 1. *The source of the transmissible plasmids used in this study*

Plasmid	Name or no. of source strain	References
F	*E. coli* K 12 58-161 or K 12 W 1655	Hayes (1952*a*), Lederberg & Lederberg (1953)
F′ lac	K 12 W 1655 F′ lac	Scaife and Gross (1963)
Col V2	K 94	Frédéricq (1963) } Macfarren &
Col V3	K 30	Frédéricq (1963) } Clowes (1967)
Col B1	CA 18	Frédéricq (1965) }
Col B2	K 77	Frédéricq (1948) }
Col B3	K 166	Frédéricq (1948) } Hausmann (1967)
Col B4	K 98	Frédéricq (1957) }
Col VB	K 260	Frédéricq 1965)
Col Ib drd	*Shigella sonnei* P 9	Ozeki, Stocker & Smith (1962), Ohki & Ozeki (1968)
R1 drd 19 R192 drd F7 R64 drd 11 R144 drd 3	*E. coli* K 12 J53 R+ derivatives	Meynell & Datta (1967)

in addition to *F* elicited suppression, the phenomenon seemed to offer a highly selective method of isolating stable *Hfr* strains and has been used, in this investigation, to assess the ability of a range of transmissible plasmids to integrate into the chromosome.

Since integrative suppression increases the apparent frequency of reversion to thermostability over that due to mutational reversion at the initiator site, an increase in the proportion of mutant bacteria capable of forming colonies at the restrictive temperature, following infection with a particular plasmid, may be used as an *a priori* criterion of plasmid integration (Nishimura *et al.* 1971). Of course the absence of such an increase does not exclude integration, for reasons that will be discussed later.

2. MATERIALS AND METHODS

The temperature-sensitive initiator mutant *CRT46* isolated from the *E. coli* K 12 subline *CR34* by Hirota *et al.* (1968) has the genotype *thr leu thi thyA ilv lac mal* ts_{DNA} F^-, and is unable to form colonies on complete medium at 42°. It was infected with the transmissible plasmids listed in Table 1. All the infections and subsequent incubation of the selective plates were performed at 30°. No difficulty was experienced in the infection of *CRT46* but the donor strains formed, and also the integratively suppressed revertants, are very difficult to maintain either on plates, slants, in stabs, or frozen in dimethyl sulphoxide (DMSO) at −70 °C.

The experimental protocol to measure integrative suppression was essentially the same as that of Nishimura *et al.* (1971). Broth cultures of the various *CRT46* donor strains, supplemented with thymine, were grown overnight at 30 °C with good aeration; they were then diluted and plated on complex medium supplemented with thymine, and prewarmed to either 30 or 42 °C. The plates were immediately incubated at 30 or 42 °C respectively in fan-assisted incubators. Colonies were counted after 24 h at 30 °C and 18 h at 42 °C.

The integratively suppressed revertants grow very poorly in liquid cultures, making the preparation of log phase cultures for fertility testing very difficult. The donor status of the initiation revertants was assessed by the following plate-mating technique: colonies to be tested were patched on triplicate master-plates (50 patches per plate) and the plates incubated overnight at 42 °C. These master-plates were replica-plated to dupli-

Table 2. *The viable counts of CRT46 donor strains* at 30 and 42 °C*

Sex-factor class	Sex factor	Viable count/ml at 30°	Viable count/ml at 42°	Ratio 42/30 °C
None	None	$4{\cdot}4 \times 10^8$	$8{\cdot}8 \times 10^2$	$2{\cdot}0 \times 10^{-6}$
F-like	F	$2{\cdot}1 \times 10^8$	$1{\cdot}3 \times 10^4$	$6{\cdot}3 \times 10^{-5}$
	F′ lac	$1{\cdot}8 \times 10^8$	$3{\cdot}6 \times 10^5$	$2{\cdot}0 \times 10^{-3}$
	Col V2	$3{\cdot}9 \times 10^8$	$1{\cdot}1 \times 10^4$	$2{\cdot}8 \times 10^{-5}$
	Col V3	$4{\cdot}2 \times 10^8$	$1{\cdot}0 \times 10^4$	$2{\cdot}3 \times 10^{-5}$
	Col B1	$1{\cdot}9 \times 10^8$	$5{\cdot}0 \times 10^3$	$2{\cdot}6 \times 10^{-5}$
	Col B2	$2{\cdot}8 \times 10^8$	$5{\cdot}1 \times 10^3$	$1{\cdot}8 \times 10^{-5}$
	Col B3	$1{\cdot}1 \times 10^8$	$1{\cdot}3 \times 10^4$	$1{\cdot}1 \times 10^{-4}$
	Col B4	$4{\cdot}1 \times 10^8$	$4{\cdot}2 \times 10^3$	$1{\cdot}1 \times 10^{-5}$
	Col VB	$1{\cdot}1 \times 10^8$	$2{\cdot}0 \times 10^3$	$1{\cdot}8 \times 10^{-5}$
	R1 drd 19	$2{\cdot}9 \times 10^8$	$1{\cdot}0 \times 10^4$	$3{\cdot}4 \times 10^{-5}$
	R192 drd F7	$1{\cdot}5 \times 10^8$	$8{\cdot}0 \times 10^3$	$4{\cdot}1 \times 10^{-5}$
I-like	Col Ib drd	$1{\cdot}5 \times 10^8$	$3{\cdot}0 \times 10^2$	$2{\cdot}0 \times 10^{-6}$
	R64 drd 11	$2{\cdot}2 \times 10^8$	$4{\cdot}6 \times 10^2$	$2{\cdot}1 \times 10^{-6}$
	R144 drd 3	$2{\cdot}5 \times 10^8$	$5{\cdot}0 \times 10^2$	$2{\cdot}0 \times 10^{-6}$

* The *CRT46 F*$^-$ strain was included in all the experiments as a control. The viable counts shown represent the average of several counts, which showed very little variation in separate experiments.

cate plates containing the same medium and the replicas were incubated for a further 3 h at 42 °C to obtain fresh log phase patches. The duplicate replica plates were then plate-mated on each of three supplemented minimal plates previously spread with 0·2 ml of a log phase broth culture of an appropriate *F*$^-$ recipient. The recipient strains were chosen to select for the transfer of donor markers widely spaced on the chromosome; they were J62 F$^-$ (*proA*$^+$, *his*, *trp*; *proA his*$^+$ *trp*; *proA his*, *trp*$^+$ selected), or *AB1157F*$^-$ (*Thr*$^+$ *leu*$^+$ *thi proA his argE*; *Thr leu thi proA his argE*$^+$ selected) and *X44F*$^-$ (*tyr*$^+$ *thi his trp purB* selected). Only when similar numbers of recombinants were given by the duplicate plate-matings for each of the three recombinant classes scored were the results regarded as valid.

3. RESULTS

(*a*) *The survival ratio at 42 and 30 °C.* The defective DNA initiation of *CRT46* was not complemented by any of the transmissible plasmids when the plasmid remained autonomous in the cytoplasm.

The viable counts obtained at 30 and 42 °C are shown in Table 2. The survival ratio, i.e. the ratio viable counts at 42°/viable counts at 30 °C, is also shown. However, this ratio cannot be used to indicate absolute frequencies of suppression because it is difficult to know the number of bacteria per plate when the revertants grow up into distinguishable colonies. There could be considerable residual cell division in these mutant strains. The survival ratio serves merely as an indication of the frequency of integration events.

It appears that all the *F*-like transmissible plasmids are capable of some degree of integrative suppression and therefore integration into the chromosome.

(*b*) *The isolation of Hfr-type donor strains.* The integratively suppressed revertants grow very slowly in liquid cultures, having a doubling time in broth of between 60 and 110 min at 42 °C. Their donor status was investigated as described in Materials and Methods. Three recombinant-classes were examined per plate-mating in order to obtain some idea of the polarity of chromosome transfer as well as fertility. A total of six

recombinant classes (*Thr*$^+$ *leu*$^+$, *pro*$^+$*A*, *trp*$^+$, *his*$^+$, *tyr*$^+$ and arg$^+$E) were examined per integratively suppressed donor strain.

All the F-like *CRT46* donor strains yield temperature-insensitive revertants at 42 °C which are highly fertile and so far as can be judged from plate-matings are *Hfr*-type donors, transferring their chromosomes in an orientated, sequential manner. Unfortunately, they are not particularly useful donor strains since, because of their long doubling time, they are rapidly overgrown by true ts^+_{DNA} revertants.

The majority of the highly fertile integratively suppressed strains appear to have the transmissible plasmids integrated in the same general region, between about 64 and 74 min on the standard chromosome map of *E. coli* (Taylor, 1970). The position of the sex-factor integration events is an approximation drawn from comparison of the number of recombinants for the markers *arg*$^+$*E*, *thr*$^+$ *leu*$^+$ and *tyr*$^+$ obtained with the suppressed donors and the numbers of the same recombinant classes obtained with known stable *Hfr* donor strains (P 13, P 72 and AB 313), whose 'origines' are in this region, in plate-mating experiments. This is no apparent bias in the direction of transfer of the *Hfr*-type donor.

A plate-mating analysis of 1500 temperature-resistant revertants selected by incubating *CRT46 ColIb*$^+$ *drd* at 42 °C failed to yield any evidence of increased fertility and no polarity of transfer. Since strains harbouring a newly transferred *ColIbdrd* plasmid show up a 100-fold increase in the total number of recombinants (Moody, to be published), a further 500 revertants were selected from *CRT46* newly infected with *ColIbdrd*. Again there was no increase in the survival ratio and no indication of fertile polarized transfer.

An additional 1000 revertants tested from *CRT46 R64*$^+$ *drd11* and *R144*$^+$ *drd3*, both *I*-like R-factors, showed the same result as *ColIbdrd*.

4. DISCUSSION

The highly selective technique of integrative suppression permits the isolation of *Hfr*-type donor strains from plasmid-carrying strains which are defective in the initiation of DNA replication, by selecting thermo-resistant revertants at 42 °C (Nishimura *et al.* 1971). Using this method, *Hfr*-type donors have been isolated from a number of *CRT46* derivatives harbouring *F*-like transmissible plasmids, including the sex-factor *F*, colicinogenic and resistance-transfer factors. In such fertile revertants it is believed that the plasmid has stably integrated into the bacterial chromosome and has taken over the role of initiation of bacterial DNA replication. The plasmid may be considered as having incorporated the entire chromosome into its genome and now replicates the chromosomal genes like an enormous *F*-prime factor.

However, since there is no increase in the survival ratio of *CRT46* when harbouring the three *I*-like plasmids *ColIbdrd*, *R64drd11 and R144drd3*, nor is there high fertility and polarized chromosome transfer by thermo-stable revertants of these strains, it is clear that interactions of these plasmids with the chromosome, if they occur at all, are very different from those of *F*-like plasmids. It is possible that these *I*-like plasmids *can* integrate stably by reciprocal recombination, but that these events either fail to mobilize the chromosome effectively, are unable to suppress the initiator mutation, or occur at too low a frequency to influence the apparent reversion rate.

Another possibility, which we prefer, is that these plasmids may mediate chromosome transfer by a non-reciprocal recombination event which does not give rise to a stable *Hfr* donor (Moody, 1972).

We would like to thank Professor W. Hayes for his encouragement and many helpful discussions during this work, and one of us (R.R.) for the generous use of facilities extended by Professor Hayes and the Medical Research Council.

REFERENCES

Broda, P., Beckwith, J. R. & Scaife, J. (1964). The characterisation of a new type of F-prime factor in *Escherichia coli* K12. *Genetical Research* **5**, 489–494.

Campbell, A. (1962). Episomes. *Advances in Genetics* **11**, 101–143.

Clowes, R. C. (1961). Colcine factors as fertility factors in bacteria: *Escherichia coli*. *Nature, London* **190**, 986–989.

Clowes, R. C. & Moody, E. E. M. (1966). Chromosomal transfer from recombination deficient strains of *Escherichia coli* K12. *Genetics* **53**, 717–726.

Edwards, S. & Meynell, G. G. (1969). I sex factors and chromosomal recombination in *Salmonella typhimurium*. *Genetical Research* **13**, 321–323.

Frédéricq, P. (1948). Actions antibiotique reciproques chez les Enterobacteriaceae. *Revue belge de Pathologie* **19**, Supplement 4, pp. 1–9.

Frédéricq, P. (1957). Colicins. *Annual Review of Microbiology* **11**, 7–22.

Frédéricq, P. (1963). On the nature of colicinogenic factors: a review. *Journal of Theoretical Biology* **4**, 159–167.

Frédéricq, P. (1965). Genetics of Colicinogenic factors. *Zentralblatt für Bakteriologie Parasitkunde*, Abt I, Originale **196**, 142–151.

Hausmann, C. (1967). Ph.D. Thesis, Federal University, Rio de Janeiro, Brazil.

Hayes, W. (1952*a*). Recombination in *Bact. coli* K12: unidirectional transfer of genetic material. *Nature, London* **169**, 118–119.

Hayes, W. (1953*b*). The mechanism of genetic recombination in *Escherichia coli*. *Cold Spring Harbor Symposium on Quantitative Biology* **18**, 75–93.

Hirota, Y., Jacob, F., Ryter, A., Bullin, G. & Nakai, T. (1968). On the process of Cellular Division in *Escherichia coli*. 1. Asymmetrical cell division and production of deoxyribonucleic acid-less bacteria. *Journal of Molecular Biology* **35**, 175–192.

Kahn, P. L. & Helinski, D. R. (1964). Relationship between colicinogenic factors E_1 and V and an F factor in *Escherichia coli*. *Journal of Bacteriology* **88**, 1573–1574.

Kahn, P. L. & Helinski, D. R. (1965). Interaction between colicinogenic Factor V and the Integrated F factor in an *Hfr* strain of *Escherichia coli*. *Journal of Bacteriology* **90**, 1276–1282.

Lederberg, E. M. & Lederberg, J. (1953). Genetic studies of lysogenicity in interstrain crosses in *E. coli*. *Genetics* **38**, 51–64.

Lederberg, J., Cavalli, L. L. & Lederberg, E. M. (1952). Sex compatability in *E. coli*. *Genetics* **37**, 720–730.

Macfarren, A. C. & Clowes, R. C. (1967). A comparative study of two F-like colicin factors, *Col* V_2 and *Col* V_3, in *Escherichia coli* K 12. *Journal of Bacteriology* **94**, 365–377.

Meynell, E. & Datta, N. (1965). Functional homology of the sex-factor and resistance transfer factors. *Nature, London* **207**, 884–885.

Meynell, E. & Datta, N. (1966*a*). The relation of resistance transfer factors to the F factor of *Escherichia coli* K 12. *Genetical Research* **7**, 134–140.

Meynell, E. & Datta, N. (1966*b*). The nature and incidence of conjugation factors in *Escherichia coli*. *Genetical Research* **7**, 141–148.

Meynell, E. & Datta, N. (1967). Mutant drug resistance factors of high transmissibility. *Nature, London* **214**, 885–887.

Moody, E. E. M. (1972). Chromosome transfer by autonomous transmissible plasmids: The stimulation of chromosome transfer by ultra-violet irradiation and by the process of new infection of donor strains. (In preparation.)

Moody, E. E. M. & Hayes, W. (1972). Chromosome transfer by autonomous transmissible plasmids: the role of the bacterial recombination (Rec) system. (Submitted to the *Journal of Bacteriology*.)

Nishimura, Y., Caro, L., Berg, C. M. & Hirota, Y. (1971). Chromosome replication in *Escherichia coli*. IV. Control of chromosome replication and cell division by an integrated episome. *Journal of Molecular Biology* **55**, 441–456.

Ohki, M. & Ozeki, H. (1968). Isolation of conjugation-constitutive mutants of colicin factor Ib. *Molecular and General Genetics* **103**, 37–41.

Ozeki, H. & Howarth, S. (1961). Colicine factors as fertility factors in bacteria: *Salmonella typhimurium*, strain LT 2. *Nature, London* **190**, 986–989.

Ozeki, H., Stocker, B. A. D. & Smith, S. M. (1962). Transmission of colicinogeny between strains of *Salmonella typhimurium* grown together. *Journal of General Microbiology* **28**, 671–687.

Scaife, J. & Gross, J. D. (1963). The mechanism of chromosome mobilisation by an F-prime factor in *Escherichia coli* K 12. *Genetical Research* **4**, 328–331.

Scaife, J. & Pekhov, A. P. (1964). Deletion of chromosome markers in association with F-prime factor formation in *Escherichia coli* K 12. *Genetical Research* **5**, 495–498.

Sugino, Y. & Hirota, Y. (1962). Conjugal fertility associated with resistance factor R in *Escherichia coli*. *Journal of Bacteriology* **84**, 902–910.

Taylor, A. L. (1970). Current linkage map. *Bacteriological Reviews* **34**, 155–175.