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The reversible transition of certain genes in *Staphylococcus aureus* between the integrated and the extra-chromosomal state

By M. H. RICHMOND and JOAN JOHNSTON

Department of Bacteriology, Bristol University Medical School, University Walk, Bristol, BS8 1TD

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1. INTRODUCTION

It is already well established in *Escherichia coli* that certain genes, or groups of genes, can behave as episomes—that is, they can undergo a free transition between the fully extrachromosomal state and one in which they are integrated in the bacterial chromosome (Jacob & Wollman, 1961; Broda, Beckwith & Scaife, 1964). Although this type of behaviour has been detected commonly among members of the enteric bacteria, it has never hitherto been demonstrated clearly in a Gram-positive species. This communication shows that a reversible transition of certain genes between the extrachromosomal and the integrated state can occur in some strains of *Staphylococcus aureus* at least.

Irreversible integration of certain extrachromosomal markers has already been reported in *S. aureus*. For example, Novick (1967) showed that genetic fragments derived originally from an extrachromosomal penicillinase plasmid could be integrated after irradiation of transducing phage preparations with u.v. light. In another series of experiments a rather unusual strain of *S. aureus* (strain PS 80) was used (Asheshov, 1966, 1968). In this strain the penicillinase gene complex is incontrovertably part of the bacterial chromosome (see Asheshov (1968) for a discussion of this point), but other markers—notably those conferring resistance to cadium ions (Cd^r) and to mercury ions (Hg^r)—are carried extrachromosomally. Normally, in transduction experiments using this strain as donor, the two groups of genes (pen^r on the one hand and $Cd^r.Hg^r$ on the other) are transferred to the recipient separately, and at very different frequencies, as would be anticipated from their separate genetic locations in the donor strain. But as a rare event it was found that both groups of markers could be transferred as a single event to form a recombined ($pen^r.Cd^r.Hg^r$) plasmid in the recipient (Asheshov, 1968). The implication of these experiments, therefore, was either that the pen^r marker could leave the chromosome to associate with $Cd^r.Hg^r$ in the plasmid state, or vice versa, in strain PS 80. The experiments do not, however, prove the free reversibility of this process.

In the experiments described in this paper, advantage has been taken of Novick's (1967) experiments which allowed the irreversible integration of a fragment of a penicillinase plasmid into the chromosome to be obtained. In this case the donor

plasmid originally carried the markers (*pen*^{r}.*Cd*^{r}.*ero*^{r}), but strains in which only the *ero*^{r} part of this group was present in the integrated state have been used. In such strains the *ero*^{r} marker is completely stable and erythromycin-sensitive segregants cannot be isolated. If, however, these strains are now transduced for a second time, this time with unirradiated phage from a donor strain carrying the plasmid markers (*pen*^{r}.*Cd*^{r}) but lacking *ero*^{r}, then transductants can be isolated that carry not only the integrated *ero*^{r} marker but also a (*pen*^{r}.*Cd*^{r}) plasmid as well. Examination of this strain shows that it can now give rise to segregants that lack *all* the markers involved in the experiment, including *ero*^{r}. The presence of a plasmid seems therefore to allow mobilization of the integrated *ero*^{r} marker so that it is lost, along with the truly plasmid markers, via the extrachromosomal state. By far the most likely mechanism for this excision to occur is one in which there is an integration of some, at least, of the plasmid markers near the *ero*^{r} region in the chromosome followed by a return to the non-chromosomal state of the plasmid markers accompanied now by the *ero*^{r} gene as well. Under these circumstances, therefore, the group of plasmid markers is behaving as a true episome.

2. METHODS

Nomenclature. The method of designating the genetic characteristics of the strains used in these experiments is based on the one described previously (Novick & Richmond, 1965). Integrated (chromosomal) markers are included between the strain number and the bracketed plasmid designation. Thus strain 8325.*ero*^{r} (α.*i*$^-$*p*$^+$.*Cd*^{r}) indicates strain 8325 carrying an *ero*^{r} marker in the chromosome, and the penicillinase *i* and *p* genes together with the marker conferring resistance to Cd^{2+} ions as part of the extrachromosomal α plasmid. The term *pen*^{r} is sometimes used as an abbreviation for the complex of regulatory and structural genes that specifies the synthesis of inducible penicillinase, and that is responsible for penicillin resistance in these strains of *Staphylococcus aureus* (Richmond, 1968).

Strains. The plasmid-less erythromycin-resistant strain 8325.*ero*^{r} (N) was isolated as described by Novick (1967). The chromosomal location of the *ero*^{r} marker was checked by the methods described by Novick (1963, 1967) and by Asheshov (1966) (see Results Section). Strain 8325.*ero*^{r} (α.*pen*^{r}.*Cd*^{r}) was constructed by transducing strain 8325.*ero*^{r} (N) with phage propagated on strain 8325 (α.*i*$^-$*p*$^+$.*Cd*^{r}.*ero*^{s}) (Richmond, 1966), and selecting the transductants on plates containing cadmium acetate. The chromosomal location of the *ero*^{r} marker and the extrachromosomal state of the *pen*^{r} and *Cd*^{r} regions in this strain was also confirmed by the methods of Novick (1963, 1967) and Asheshov (1966) (see Results Section).

Transduction experiments. Transduction of genetic markers was carried out essentially as described by Novick (1963). In all the experiments described here, transductants were selected on agar containing 10^{-4} M cadmium acetate.

Irradiation of transducing phage. Phage preparations in CH medium (Novick, 1963) were irradiated for various lengths of time using a Hanovia Model 11 lamp as u.v. sourve. The distance between source and sample was 30 cm.

Media. Growth media and the conditions of culture of the strains used here have been described previously by Novick (1963).

3. RESULTS

The chromosomal location of the ero^r *region in strains 8325*.ero^r (*N*) *and in strain 8325*.ero^r (pen^r.Cd^r).

The chromosomal location of the *ero^r* marker in both these strains and the extrachromosomal state of the (*pen^r.Cd^r*) plasmid in the second of the two was checked by the methods described by Novick (1963, 1967) and by Asheshov (1966, 1968). The technique used by these authors was originally developed by Arber (1960) to

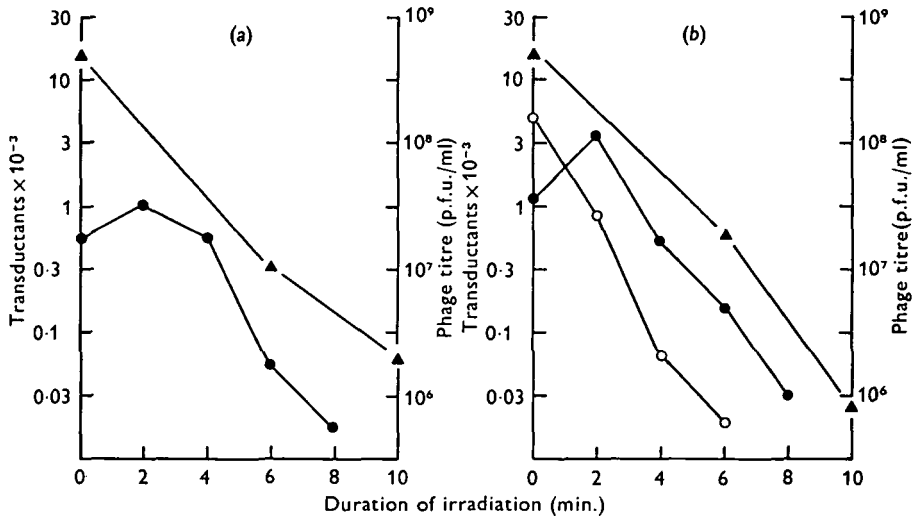

Fig. 1. The effect of ultraviolet irradiation on the transduction of genetic characters from two strains of *Staphylococcus aureus* and also on the plaque-forming ability of the transducing phages. (*a*) Donor strain: 8325.*ero^r* (N). Erythromycin resistant (*ero^r*) transductants, (●); plaque-forming ability of transducing phage preparation, (▲). (*b*) Donor strain: 8325.*ero^r*(*α*.*pen^r*.*Cd*). Erythromycin resistant (*ero^r*) transductants, (●); penicillin resistant and cadmium ion resistant, (*pen^r*.*Cd^r*), transductants, (○); plaque-forming ability of transducing phage preparation (▲).

study the location of chromosomal and extrachromosomal markers in *Escherichia coli* and depends upon the effect of ultraviolet irradiation of transducing phage on the frequency of gene transfer by the phage. In the case of chromosomal markers, there is a marked stimulation of transducing efficiency after irradiation with low u.v. doses—sometimes as much as 50-fold whereas—with an extrachromosomal gene there is an exponential decay in transducing efficiency which decreases in parallel to phage plaque-forming ability. According to the test, therefore, one would expect a stimulation of the ability to transfer the *ero^r* marker from both strains on u.v. irradiation, but an exponential fall in the transduction of the *pen^r* and *Cd^r* genes from strain 8325.*ero^r* (*pen^r*.*Cd^r*). Examination of the effect of u.v.

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irradiation on the frequency of transduction of the *ero*^{r} marker from strain 8325.*ero*^{r} (N) and of the *ero*^{r}, *pen*^{r} and *Cd*^{r} markers from strains 8325.*ero*^{r} (*pen*^{r}.*Cd*^{r}) is shown in Fig. 1. Whereas the transduction frequency of the *ero*^{r} marker from both strains is stimulated by low doses of irradiation before decreasing at higher doses, the transfer of the (*pen*^{r}.*Cd*^{r}) markers from strain 8325.*ero*^{r}.(*pen*^{r}.*Cd*^{r}) decays exponentially as irradiation proceeds. These results therefore confirm the chromosomal location of the *ero*^{r} marker in both strains and the plasmid location of the (*pen*^{r}.*Cd*^{r}) group of genes in strain 8325.*ero*^{r}(*pen*^{r}.*Cd*^{r}).

The segregation patterns of strains 8325.ero^r (*N*) *and 8325*.ero^r (α.pen^{r}Cd^r)

In order to compare the segregation pattern of these two strains, single colonies of each growing on the surface of non-selective agar were picked into fresh 100 ml quantities of CH medium, emulsified, and grown aerobically until the cultures had reached a density of about 10^8 bacteria/ml. At this point, samples were

Table 1. *Segregation patterns of strains 8325*.ero^r (*N*) *and 8325*.ero^r (α.pen^r.Cd^r)

Parental strain...	8325.*ero*^{r} (N)		8325.*ero*^{r} (α.*pen*^{r}.*Cd*^{r})*	
	No.	%	No.	%
Total cells examined	4800	100	2950	100
Segregants				
8325–*ero*^{r} (N)	—	—	12	0·41
8325 (α.*pen*^{r}.*Cd*^{r})	—	—	0	0
8325 (N)	0	0	16	0·54

* In this experiment, the incidence of strain 8325.*ero*^{r} (α.*pen*^{r}.*Cd*^{r}) among the segregants could not be distinguished from that of strain 8325 (α.*pen*^{r}.*Cd*^{r}.*ero*^{r}).

removed and plated on plain agar to give 200–500 colonies on each plate. After these colonies had grown to a suitable size, they were replicated onto plain agar, onto agar containing 10 μg erythromycin/ml and also onto agar containing 10^{-4} M cadmium acetate to discover the resistance pattern of the segregants. In particular it was important to determine the proportion of erythromycin-sensitive segregants arising from the two types of culture. After growth of the replica plates, the presence of the *pen*^{r} marker was determined by staining the plates with the penicillin + iodine reagent (Novick & Richmond, 1965) to detect the presence of penicillinase. The results of this experiment (Table 1) show that whereas no erythromycin-sensitive segregants arose from strain 8325.*ero*^{r} (N), 0·54 % of the segregants arising from strain 8325.*ero*^{r} (α.*pen*^{r}.*Cd*^{r}) lacked not only the *ero*^{r} marker but *pen*^{r} and *Cd*^{r} as well. Moreover, no segregants of the genotype 8325.*ero*^{s}.(α.*pen*^{r}.*Cd*^{r}) were found, which suggested that the loss of the *ero*^{r} marker cannot occur without the concomitant loss of the (α.*pen*^{r}.*Cd*^{r}) plasmid; that is, that an associa-

tion between the integrated *ero*^{r} marker and the plasmid must have been formed en route to the final loss of *ero*^{r} from the cell.

4. DISCUSSION

The fact that the integrated *ero*^{r} marker can be lost from strain 8325 when there is a plasmid in the cell but not otherwise, and, moreover, that the loss of *ero*^{r} is always accompanied by the concomitant loss of the plasmid markers *Cd*^{r} and *pen*^{r}, argues strongly that an essential preliminary step in the mobilization of *ero*^{r} by the plasmid is a recombination event linking *pen*^{r}.*Cd*^{r} and *ero*^{r}. The most likely mechanism by which this process could occur was originally suggested by Campbell (1962) to explain the integration of a phage genome to the prophage state and subsequently has been shown to account for the reversible integration of the *lac* region in a strain of *Escherichia coli* carrying a *F′.lac* (Broda, *et al.* 1964). The Campbell model requires—at least in the form proposed by him—that the non-chromosomal element is circular, and there is now a considerable amount of evidence to suggest that this is the case for the penicillinase plasmids—at least at some stage of their existence. First, the genetic interactions involved in the 'association' of two plasmids (Richmond, 1967) can most easily be interpreted if a circular conformation for the interacting plasmids is invoked, and secondly unpublished experiments by R. P. Novick (personal communication) have shown that the series of partial genetic deletions which have been obtained can only be ordered unambiguously if the linkage map of the penicillinase plasmids is circular. In this respect, therefore, the penicillinase plasmids of *S. aureus* are similar to other extrachromosomal elements, such as the F-like and I-like plasmids of the enteric bacteria (Meynell, Meynell & Datta, 1968).

According to the Campbell hypothesis, the mobilization of *ero*^{r} by the plasmid genes in these experiments with *S. aureus* would occur as follows (Fig. 2):

(1) Apposition of the homologous regions in the plasmid and the chromosome (*A* with *a* and *B* with *b*, Fig. 2).

(2) The formation of a single cross-over in one of the two homologous regions (shown in *A*–*a* in Fig. 2).

(3) The formation of a single structure in which the plasmid-linked genes are now part of a single covalently linked structure with the chromosome; that is they have become integrated.

(4) Apposition of the two copies of the second homologous region (*B*/*b*, Fig. 2).

(5) Re-formation of an extra-chromosomal element now carrying an *ero*^{r} marker in addition to the *pen*^{r} and *Cd*^{r} markers carried originally.

The process depends on the presence of two genetic regions on the plasmid homologous with two regions on the chromosome, one lying on each side of the integrated *ero*^{r} region (see *A*/*a* and *B*/*b* in Fig. 2). The reasons for believing in the presence of these are as follows:

(1) There is genetic homology between the plasmids (*pen*^{r}.*Cd*^{r}.*ero*^{r} and *pen*^{r}.*Cd*^{r}) since they have genetic markers in common. Furthermore, as penicillinase plasmids

may behave as though they are circular (see Richmond (1967) and above), some of this homology must lie on both sides of the *ero*^{r} marker in the plasmid (*pen*^{r}.*Cd*^{r}.*ero*^{r}).

(2) It is most unlikely that all the genetic information on a penicillinase plasmid

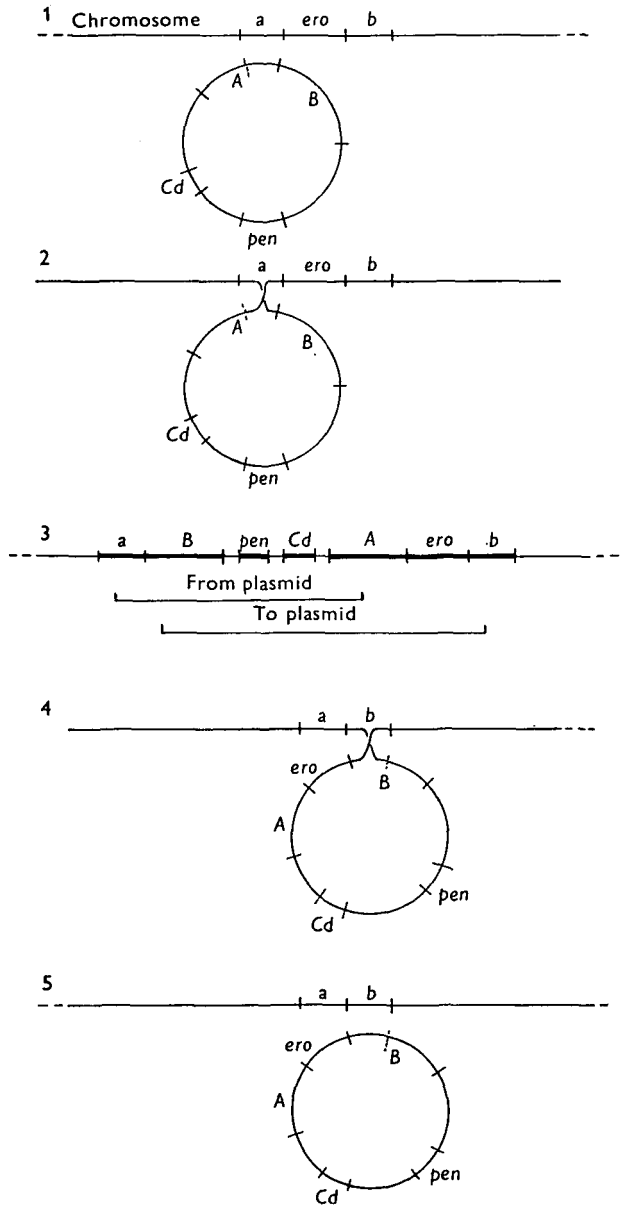

Fig. 2. A possible mechanism for the mobilization of a chromosomal *ero*^{r} region by a plasmid in the strain 8325.*ero*^{r}(*α*.*pen*^{r}.*Cd*^{r}). (1) Apposition of region *A* on the plasmid with region *a* on the chromosome. (2) Insertion of the plasmid by means of a single cross-over in the *A*/*a* region (3) The product of step 2. (4) Excision of the *ero*^{r} region by a single cross-over in the region *B*/*b*. (5) The final situation with the *ero*^{r} region now excised. For a discussion of the origin of the regions *a* and *b*, see text.

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has yet been accounted for in terms of phenotypic characters. Thus, there may well be two genetic regions (*A* and *B*, Fig. 2) to which no phenotypic character can be ascribed.

(3) When the plasmid (α . *pen*^{r} . *Cd*^{r} . *ero*^{r}) is used to establish strain 8325 . *ero*^{r} (N) by the method described by Novick (1967), it is inconceivable that the *ero*^{r} gene alone is integrated; it is much more likely that a certain amount of genetic material from each side of the *ero*^{r} marker on the plasmid is integrated as well. One of these regions, *a*, is the fragment derived from the plasmid region *A* and similarly *b* is derived from *B* (see Fig. 2).

The results of the experiments shown in Table 1 suggest that the plasmid in strain 8325 . *ero*^{r} (α . *pen*^{r} . *Cd*^{r})—or part of it at least—is behaving as a true episome (cf. Jacob & Wollman, 1961). However, to describe penicillinase plasmids as true episomes is to oversimplify the picture to some extent. When plasmid markers undergo reversible integration, they are indeed behaving as true episomes, yet plasmid genes in *S. aureus* can also 'associate' with other extrachromosomal elements (e.g. with other penicilllnase plasmids (Richmond, 1967)). This process amounts to the reversible 'integration' of one extrachromosomal element into another, not into the chromosome, and is a type of interaction not envisaged in the original definition of an episome. It appears therefore that the term episome, although originally useful to describe the behaviour of some extrachromosomal elements, is not too restrictive a term to describe the full versatility of the genetic interactions of some bacterial plasmids (cf. Hayes, 1969).

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