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REVIEW

Plasmids That Mobilize Bacterial Chromosome

B. W. HOLLOWAY

Department of Genetics, Monash University, Clayton, Victoria 3168, Australia

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I. INTRODUCTION

With much of the current interest in plasmids centered on their antibiotic resistance properties, their role in genetic engineering, or their use in studying DNA replication, it is worth remembering that the first plasmid to be studied was of interest to bacterial geneticists because it had the property of transferring the *Escherichia coli* K12 chromosome in conjugation and could be used for the formal genetic study of that organism. With the passage of time, it is apparent that this ability is also possessed by other plasmids found in widely different genera of bacteria. An important consequence is that the advantages of genetic analysis by conjugation are now available for the study of a wide variety of bacteria. The techniques of *in vitro* genetic engineering have permitted the isolation of unusual recombinants containing genetic information from quite unrelated organisms. The ability of some plasmids to promote chromosome transfer between different bacterial genera promises to provide an alternative set of procedures for achieving such unusual recombinants, being referred to by some authors as *in vivo* or natural genetic engineering (Reanney, 1976).

The phenotypic ability of plasmids to transfer host chromosome has been denoted chromosome mobilization ability and given the symbol Cma (Haas and Holloway, 1978). It is the purpose of this review to examine the extent of Cma among different bacteria, to discuss the mechanisms by which plasmids can carry out this process, including the role of the host bacterium, and to indicate the advantages that may accrue from the study of Cma in a variety of bacteria.

II. THE OCCURRENCE OF Cma IN BACTERIAL GENERA

Table 1 provides a listing of plasmids that can transfer host chromosome in different bacteria. It was convenient in the construction of this table to distinguish two types of bacterial chromosome transfer by plasmids. The examples in Table 1 include all those situations in which transfer of chromosome is not necessarily accompanied by inheritance of the whole plasmid. The second type, which encompasses the F prime (F′) and R prime (R′) examples, involves the formation of a hybrid plasmid comprising both plasmid and bacterial DNA in which all or most of the plasmid functions have been preserved. These will be discussed in a separate section below.

From the data in Table 1 it is clear that Cma is a common property among plasmids and, for various reasons, the list in Table 1 is probably incomplete. For example, as

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TABLE 1

BACTERIA AND PLASMIDS SHOWING Cma[a]

Bacteria and plasmids	Comments	References
Acinetobacter calcoaceticus	Only IncP-1 plasmids (as listed) shown to have Cma, recombinant formation being 10^{-6}–10^{-8}/recipient cell depending upon the marker selected. No marked evidence for oriented transfer of markers. RP4 used to map 23 loci on a circular linkage map.	Towner (1978) Towner and Vivian (1976a,b, 1977)
RP4*		
R751*		
R_{GN823}*		
R702*		
R1033*		
Citrobacter freundii		
F	Oriented transfer similar to *E. coli* Hfr strains.	deGraff *et al.* (1974)
Escherichia coli		
F	Chromosome transferred following stable integration (Hfr form) or by F^+ form with possible transient integration. Oriented transfer, genetic circularity of the chromosome established.	Lederberg *et al.* (1952) Jacob and Wollman (1961) Hayes (1968)
ColV	Plasmid can integrate stably into the chromosome to produce Hfr form.	Kahn (1968)
ColI	No evidence for chromosomal integration. Cma independent of donor *rec*A^+ function. No oriented transfer.	Clowes (1961) Clowes and Moody (1966)
Other F-like plasmids	Only Cma demonstrated, no linkage data available.	Bernstein (1968)
R68.45*	No published details of marker transfer.	Kondorosi *et al.* (1977) Beringer and Hopwood (1976)
R1*drd*19 R179II	Derepressed mutant shows higher marker transfer frequency than wild-type plasmid. Oriented high frequency transfer of *trp* region.	Pearce and Meynell (1968)
R179II	Oriented transfer from a site between *arg* and *pro* on the *E. coli* C chromosome.	Hedén and Rutberg (1976)
R538-2*drd* (and other R plasmids)	All markers examined transferred at same frequency, no evidence for oriented transfer.	Cooke and Meynell (1969) Sugino and Hirota (1962)
Erwinia chrysanthemi		
F'*lac*$^+$	Indirect evidence of F'lac$^+$ integration into the chromosome with oriented transfer from a region near *leu*.	Chatterjee and Starr (1977)
Erwinia amylovora		
F'*lac*$^+$	Indirect evidence of F'lac$^+$ integration into the chromosome with oriented transfer from a site different from that found with *Erwinia chrysanthemi*.	Chatterjee and Starr (1973)
Klebsiella pneumoniae		
R144*drd*3	Marker transfer frequencies of ca. 10^{-5}. With donor strain HF3, but not with other donor strains, oriented transfer occurred.	Dixon *et al.* (1975)
Pasteurella pseudotuberculosis	Transfer of some markers only. Insufficient data to indicate oriented transfer.	Lawton *et al.* (1968)

TABLE 1 (*Continued*)

Bacteria and plasmids	Comments	References
Proteus mirabilis		
P-lacR1*drd*19 (D)	Marker transfer frequencies vary from 5×10^{-6}/donor parent to less than 10^{-8}. Oriented transfer from one origin site. Chromosome circularity not established.	Coetzee (1975, 1978a)
R772*	Marker transfer frequencies of ca. 5×10^{-5}/donor parent for all markers tested. Evidence for multiple sites of entry and transfer of short segments of donor chromosome. R772 is stable in chromosomal recombinants unlike R68.45 in *P. aeruginosa* PAO.	Coetzee (1978b)
Pseudomonas aeruginosa		
FP2	Displays Cma in strains PAO and PAT with one transfer origin only and clockwise oriented transfer. No detectable stable chromosomal integration. Possesses a mercury resistance determinant.	Holloway *et al.* (1971) Loutit (1970) Watson and Holloway (1978)
FP39	Displays Cma in strain PAO, with one transfer origin 10 min distal to that for FP2 oriented transfer (clockwise) and no stable chromosomal integration detected. Can complement one class of leucine auxotrophs in strains PAO and PAT.	Pemberton and Holloway (1973)
FP5	Displays Cma in strain PAO, with one transfer origin the same as FP2, oriented transfer (clockwise), and no stable chromosomal integration. Does not show entry exclusion with FP2. Unlike FP2 or FP39, Cma can be cured by acriflavin.	Matsumoto and Tazaki (1973)
FP110	Displays Cma in strain PAO, with one transfer origin ca. 25 min proximal to that for FP2. Oriented transfer in an anti-clockwise direction. FP′ plasmids have been derived from this plasmid.	Royle and Holloway (unpublished data)
R68*	Efficient Cma (up to 10^{-3}/donor parent) in strain PAT but ineffective in strain PAO ($<10^{-8}$/donor parent). Oriented transfer (clockwise) and one transfer origin in strain PAT.	Stanisich and Holloway (1971) Watson and Holloway (1978)
R91-5	Derepressed mutant of R91 (IncP-10) with oriented transfer and two transfer origins in strain PAT. Has Cma in strain PAO but not extensively studied. Does not show Cma in *Acinetobacter calcoaceticus*.	Stanisich and Holloway (1971) Watson and Holloway (1978)
R68.44* R68.45*	Variants of R68 with enhanced Cma for PAO. Also has Cma in PAT and PAC. Multiple transfer origins. R68.45 is a more stable variant of R68.44. Retains wide host range of R68 (see other genera in this table).	Holloway (1975) Haas and Holloway (1976, 1978)
pND2	Isolated in *P. arvilla* as the plasmid TOL conferring ability to grow on toluate. Shows Cma in strain PAO, with evidence for multiple origins.	White and Dunn (1977)
Pseudomonas glycinea		
R68* RP1*	Marker transfer frequencies from 10^{-6}–10^{-9}/donor parent. No linkage of any markers demonstrated.	Lacy and Leary (1976)

TABLE 1 (*Continued*)

Bacteria and plasmids	Comments	References
Pseudomonas putida		
R68.45*	Transfer frequencies of up to 10^{-5}/donor parent with multiple origins likely.	Martinez and Clark (1975) Morgan, Dean, and Holloway (unpublished data)
XYL-K	K is part of the OCT plasmid and has Cma. K is unstable, but XYL-K hybrids are stable when selection is made for XYL on xylene containing media. XYL is a conjugative xylene degradative plasmid. Interrupted matings indicate one transfer origin and oriented transfer. A map with 25 markers has been prepared but circularity has not been demonstrated.	Mylroie *et al.* (1977)
pfdm	Derived from transducing bacteriophage *pf*16*h*2, has low level Cma (10^{-9}/donor parent), and is thought to be a bacteriophage–bacterial chromosome hybrid.	Chakrabarty and Gunsalus (1969)
Rhizobium leguminosarum		
R68.45*	Recombinant frequency ca. 10^{-6}/donor parent. Linkage data suggest multiple sites of origin, and proportionally larger fragments of chromosome mobilized than with R68.45 in *P. aeruginosa* PAO. A circular linkage map with 17 markers has been constructed. Interspecific crosses (*R. leguminosarum* × *R. meliloti*) were fertile at ca. 1–10% of intraspecific crosses by either haploid recombinant formation or by the production of R′ plasmids. Other interspecific crosses are fertile, the level of recombinants depending on the species involved and the markers selected.	Beringer and Hopwood (1976) Johnston and Beringer (1977) Beringer *et al.* (1978) Johnston *et al.* (1978)
Rhizobium meliloti		
R68.45*	Recombinant frequency ca. 10^{-3}–10^{-5}/donor cell. A circular chromosome map having 19 markers has been constructed. Linkage data suggest multiple sites of origin and also that ca. 30% of the chromosome may be the average donor chromosome fragment transferred. Most of the markers used in *R. meliloti* and *R. leguminosarum* crosses involving R68.45 are auxotrophic and lack of knowledge of the enzyme deficiencies involved prevents a comparison of the chromosome maps of these species at present.	Kondorosi *et al.* (1977)
RP4*	Transfer frequency of all markers tested is similar varying from 10^{-5}–10^{-6}/donor parent. No evidence for oriented transfer. Twenty markers have been mapped and can be arranged in a circular configuration.	Meade and Signer (1977)
Rhodopseudomonas sphaeroides		
R68.45*	Marker transfer frequencies of 10^{-4}–10^{-8}/donor parent. Some evidence of marker linkage.	Sistrom (1977)
Salmonella typhimurium		
F	Chromosomal integration of plasmid with oriented transfer.	Zinder (1960) Levinthal and Sanderson (1974)

TABLE 1 (*Continued*)

Bacteria and plasmids	Comments	References
R1*drd*	Oriented transfer from *trp* region, Cma is *rec*A independent and can be lost from the plasmid without affecting other properties of the plasmid.	Heden and Meynell (1976)
*col*I		Ozaki and Howarth (1961)
Other *Salmonella* species		
F	Chromosomal integration of plasmid with oriented transfer.	Levinthal and Sanderson (1974)
Serratia marcescens		
R471 R477b R1P69	Marker transfer frequencies vary from 10^{-5}–10^{-8}/donor parent. No evidence of linkage.	Hedges (1978)
Streptococcus faecalis		
pAMγ1 pAMγ2 pAD1 pOB1	Marker transfer frequencies vary from 10^{-6}–10^{-8}/donor parent. Linkage of chromosomal antibiotic resistance markers demonstrated.	Franke *et al.* (1978)
Streptomyces coelicolor		
SCP1	The plasmid can interact at various sites on the chromosome and promote chromosome transfer bidirectionally or unidirectionally. It carries genes for methyenomycin synthesis and resistance and can be transferred to other species of *Streptomyces*. SCP1 can form chromosome plasmid hybrids. Genetic circularity of the chromosome established.	Hopwood and Wright (1976)
SCP2	Found in association with SCP1 and responsible for aspects of chromosome transfer in strain A3(2). A variant SCP2* can promote enhanced generalized recombination apparently from multiple sites on the chromosome.	Bibb *et al.* (1977)

[a] The data for *E. coli* and *Salmonella* have been extensively documented elsewhere and the treatment of these bacteria is minimal in this table. Further details can be obtained by reference to the articles referred to in the text. IncP-1 plasmids are indicated with an asterisk.

some plasmids, e.g., ColV or R64, show an oriented transfer from only one origin, unless a range of markers is tested, in any given case such oriented transfer might be missed. Structural or hereditary plasmid instability (see below) occurs in some Cma plasmids [e.g., K in *P. putida* (Mylroie *et al.*, 1977), R68 in *P. aeruginosa* strain PAT (Haas and Holloway, 1976)] and, unless selection can be imposed for the plasmid, as accomplished by Mylroie *et al.* by isolating XYL-K recombinant plasmids for which selection for growth on xylene can be made, the Cma properties of the plasmid may be lost. If the plasmid is naturally repressed for transfer then Cma frequency may be too low for detection. Plasmid mutants depressed for transfer show effective levels of Cma (Cooke and Meynell, 1969).

There are a number of bacterial genera for which systems of conjugative transfer of chromosome have been demonstrated and mapping of genes accomplished but for which insufficient evidence is available to im-

plicate a plasmid as an essential component of the chromosomal mobilization mechanism. These include *Rhizobium lupini* (Heuman, 1968; Heuman and Springer, 1977), *Klebsiella pneumoniae* (Matsumoto and Tazaki, 1970, 1971), and *Vibrio cholerae* (Bhaskaran and Iyer, 1961). Other genera have been referred to in a review by Levinthal (1974).

Perhaps the widest survey of plasmids for the occurrence of Cma has been carried out in *P. aeruginosa* (Pemberton and Holloway, 1973; Holloway *et al.*, 1978; Royle and Holloway, unpublished data; Hindman and Morgan, unpublished data). One of the motivations for this work has been the desire to test the *P. aeruginosa* strain PAO chromosome for genetic circularity. The first plasmids used for mapping in this organism, FP2 and FP39, each had a single transfer origin; the sites of these origins did not allow a definite test of chromosomal circularity. Most other plasmids isolated in this organism had the same transfer origin as FP2 (see Holloway, 1975, for a chromosome map of *P. aeruginosa)*. Stanisich and Holloway (1971) showed that various R plasmids possess Cma and recombinants for chromosomal markers can be recovered at frequencies higher than that found for FP2. Using FP2, R68, R91-5, and R68.45, Watson and Holloway (1978) have recently demonstrated genetic circularity for the *P. aeruginosa* strain PAT but this has not yet been accomplished for strain PAO.

Several features of the data in Table 1 are worthy of special mention. First, a gram-positive coccus, *Streptococcus pyogenes*, appears on such a list for the first time and elaboration of these preliminary observations will be awaited with interest. Second, the IncP-1 plasmids, including RP4, R68, R68.45, and others, possessing the ability to establish themselves in a wide range of gram-negative bacteria, as well as having Cma, constitute an important class of plasmids of advantage to bacterial genetics in general and to the study of Cma in particular. The diversity of plasmids and organisms in which Cma is found raises the possibility that there may be more than one mechanism by which plasmids cause host chromosome to be transferred in conjugation.

III. THE MECHANISMS OF CHROMOSOME TRANSFER

The best, and probably only, example of plasmid-mediated Cma for which the mechanism of chromosome transfer has been established is that of F in *Escherichia coli*. It has been established that F can integrate stably into the *E. coli* chromosome (for reviews see Jacob and Wollman, 1961; Hayes, 1968; Curtiss, 1969) at a variety of sites (Matney *et al.*, 1964; Curtiss *et al.*, 1974) to create Hfr strains each having a specific transfer origin and direction. Chromosome transfer is largely dependent upon a competent recombination system (*rec*A^+) in $F^- \times F^+$ matings but *rec* A^+ is not required for chromosome mobilization and transfer in Hfr donors. Integration of F into the chromosome is partially *rec* A^+ dependent as shown by studies with *rec*A F′ strains (Wilkins, 1969).

It is known that the DNA transferred in conjugation in *E. coli* is single stranded (for review see Willetts, 1972; Achtman and Skurray, 1977), and, although the enzymatic basis of transfer is not known, DNA replication commences from a site *ori* T, the origin of DNA transfer, mapping at 62 kb on the F genome, and continues beyond the boundaries of that genome into the bacterial genome. Through the function of other genes of the F plasmid this leads to bacterial DNA transfer to the recipient cell (Curtiss and Fenwick, 1975; Willetts, 1972; Willetts *et al.*, 1976; Achtman and Skurray, 1977; Clark *et al.*, 1976).

Despite the extensive body of information available for the *E. coli*-F system, we still do not know the precise molecular mechanism by which chromosome is mobilized by this plasmid. It is also very likely that the

formation of stable Hfr strains, as with F in *E. coli*, is not the common mechanism by which many other plasmids with Cma mobilize chromosome. Apart from some F-like plasmids, including ColB and ColV (Kahn, 1968), there is very little convincing evidence that any other plasmid integrates into the chromosome to form an Hfr-like donor strain.

One exception may be with those plasmids, including R plasmids and Col plasmids, which show integrative suppression (Nishimura *et al.*, 1971, 1973; Yoshimoto and Yoshikawa, 1975) but such plasmids are known to be very similar to F, and, while the evidence for polarized transfer of chromosome is acceptable, the genetic evidence of integration is not always as extensive enough to be entirely convincing. I-like plasmids also integratively suppress and give donors with oriented transfer of chromosome (Datta and Barth, 1976).

One of the key questions relating to the role of plasmids in chromosome mobilization is that of plasmid integration at the origin of chromosome transfer. Stable integration of plasmids into the bacterial chromosome is a rare phenomenon, with the exception of temperate bacteriophages. The most likely alternative is that some form of transient or unstable integration is involved. In the case of F, Curtiss and Stallions (1969) confirming earlier results of Reeves (1960) and Broda (1967), concluded that 80–85% of recombinants formed in $F^- \times F^+$ matings in *E. coli* K12 resulted from either unstable or lethal integration of F into the chromosome, or by a mechanism that did not involve F integration at all. Such transient integration very likely involves host *rec*A^+ function because in both *E. coli* (Clowes and Moody, 1966) and *P. aeruginosa* (Chandler and Krishnapillai, 1974a) recombinant formation is markedly reduced with a RecA-deficient donor. However, the existence of another pathway for chromosome mobilization is shown by the fact that detectable recombinant formation does take place in such matings with a *rec*A donor.

With the exception of the Hfr form with F and those plasmids showing integrative suppression no evidence for stable integration of plasmids as a prerequisite for chromosome mobilization has been presented for any of the examples listed in Table 1. As yet no convincing protocol for demonstrating transient integration of plasmids has been devised, although this model, rather than one that does not involve any integration, seems to be the more likely alternative. For the present, firm conclusions as to the nature and role of integration in Cma must await further data.

The type of data needed is perhaps best illustrated by the work of Davidson *et al.* (1975) and Ohtsubo and Ohtsubo (1977) who have demonstrated the involvement of insertion sequences (IS), located on both F and the *E. coli* chromosome, in the integration of F into the *E. coli* chromosome to form Hfr and F′ strains. The sites of insertion sequences have been mapped in F and it has been suggested that the ends of insertion sequences can act preferentially as recombination sites in F and R plasmids (Davidson *et al.*, 1975; Hu *et al.*, 1975; Ohtsubo and Ohtsubo, 1977). The distribution of insertion sequences on the *E. coli* chromosome has been studied (Chow, 1977) and, depending on the methods used, as many as 100 individual insertion sequences may occur on the chromosome, comprising at least six types. The general conclusion from this and other work involving insertion sequences (for review, see Bukhari, *et al.*, 1977) is that integration of plasmids into the bacterial chromosome, both transient and stable, may involve specific nucleotide sequences on both plasmid and chromosome.

IV. CONSTRUCTION OF PLASMIDS WITH Cma

The need to have systems of conjugation for genetic analysis of particular bacteria, or more recently, the interest in techniques for transfer for DNA between unrelated bacteria, has encouraged a number of workers

to try to synthesize plasmids with Cma from plasmids that do not have this ability or to extend the host range or efficiency of those that do. Such an example is found in *Proteus mirabilis* where Coetzee (1974), using phage PL25, transduced the R plasmid R1*drd*19 into Providence P29 strains harboring the transmissible plasmid P-*lac*, which can transmit the Lac$^+$ phenotype. A hybrid plasmid isolated from this transduction, P-*lac* R1*drd*19, can be transmitted at high frequency to *P. mirabilis* as well as to other enteric bacteria. As indicated in Table 1, this hybrid plasmid, now referred to as plasmid D, can mobilize the *P. mirabilis* chromosome from one origin (Coetzee, 1978a).

In one case it has even been possible to modify a bacteriophage so that it can mobilize chromosome. The bacteriophage Pf16 transduces chromosomal markers in *P. putida* (Chakrabarty and Gunsalus, 1969). A modified bacteriophage was isolated in which a portion of the genome was replaced by DNA from the *P. putida* chromosome including the region concerned with mandelate metabolism. Such bacteriophage particles, denoted *pfdm*, acquire transmission properties similar to those of plasmids and have the ability to promote bacterial chromosome transfer, albeit at low frequency. The molecular mechanisms involved in the production of *pfdm* and its mechanism of chromosome transfer are not known.

The IncP-1 plasmids have attracted particular attention in view of their wide host range–which includes a number of organisms of considerable scientific, economic, and medical significance. On the basis of the fact that, in *E. coli*, F′ plasmids can affect mobilization of the chromosome (Low, 1972), Jacob *et al.* (1976) attempted to insert a piece of bacterial DNA into an IncP-1 plasmid in the hope that it would create an efficient sex factor for the particular bacterial species from which the inserted chromosome segment had been obtained. However, although plasmids were constructed in which segments of either *Rhizobium leguminosarum* or *Proteus mirabilis* chromosome had been inserted into RP4, it was found that, while such recombinant plasmids retained the transfer characteristics and wide host range of the parental plasmid, they were no more efficient in mobilizing chromosome than the parent plasmid RP4. These plasmids in some respects resemble F′ plasmids in that they can determine a conjugation mechanism and possess a region of genetic homology between the plasmid and the host chromosome. They evidently lack the necessary mechanism to mobilize chromosome which in the case of F′ plasmids (see below) has been ascribed to insertion sequences. Insertion sequences on the plasmid are not the entire answer to this problem because RP4 can mobilize chromosome in *P. aeruginosa* PAT (Stanisich and Holloway, 1971; Holloway and Richmond, 1973).

More recently, the increased knowledge of the bacteriophage mu has encouraged a number of workers to use RP4::mu plasmids to promote bacterial conjugation and for *in vivo* genetic engineering purposes. Dénarié *et al.* (1977) and Faelen *et al.* (1977) have developed techniques for the insertion of mu into RP4 involving the use of a temperature-sensitive mutant of mu and have shown that such hybrid plasmids can be transferred to other bacteria including *Klebsiella pneumoniae, Pseudomonas solanacearum, R. meliloti, Serratia, Proteus,* and *E. coli*. It was found that the RP4::mu mobilized *E. coli* chromosome at frequencies as high as 10^{-4} per donor parent, that the transfer occurred in an oriented manner, and that chromosome mobilization is initiated at the point of the mu insertion into the chromosome. The fact that appreciable frequencies of recombinants were recovered using *rec*A recipients indicated that R′ plasmids were produced and responsible for some of the chromosome transfer observed. This experiment demonstrates that RP4 has the genetic information needed to provide the necessary mechanisms for chromosome transfer. What is needed in addition is a specific nucleotide sequence on the plasmid which can enable interaction of plasmid and bacterial

chromosome to initiate chromosome mobilization. In the RP4::mu hybrids, this can be provided by mu. Using RP4::mu *cts*62 adapted to *Klebsiella*, transfer of *Klebsiella* genes was shown in *Klebsiella* × *Klebsiella* matings. It was also possible to transfer *Klebsiella* genes to *E. coli* by this mating system.

In bacteria such as *R. meliloti* and *P. solanacearum* there is expression of the mu genome as is shown by the production of infectious particles, albeit at very low titer. Provided methods can be devised to allow selection of mu insertions into the chromosome of such bacteria, it should be possible to obtain conjugational chromosome transfer in any bacterium in which mu can express itself. Certainly the field is now open for the use of mu in a variety of Enterobacteria for such procedures as chromosome mobilization and production of hybrid plasmids containing regions of bacterial chromosome, and this should extend the range of genetic analyses possible for such important organisms as nitrogen-fixing *Klebsiella* and plant pathogenic *Erwinia*.

The expectations of the RP4::mu work have to some extent been realized by the isolation of R68.45 in *Pseudomonas aeruginosa* from the broad host range plasmid R68 (Cb^r, Tc^r, Km^r) of the IncP-1 group (Jacoby and Shapiro, 1977). It had been observed that R68, like other IncP-1 plasmids, promoted chromosome transfer in strain PAT of *P. aeruginosa* at frequencies as high as 4×10^{-3}/donor parent cell for markers close to the origin site, and, for a range of markers, transfer frequencies 2- to 10-fold higher than with FP2 are obtained (Stanisich and Holloway, 1971; Stanisich, 1972; Watson and Holloway, 1978; see Holloway, 1975, for a description of FP plasmids and *P. aeruginosa* strains PAO and PAT). However, IncP-1 plasmids were found to be ineffective in promoting chromosome transfer in *P. aeruginosa* strain PAO. A search was made for variants of various IncP-1 plasmids that would be effective in promoting chromosome transfer in strain PAO. This involved an examination of plasmids carried by those rare recombinants (10^{-8}/donor parent cell) for chromosomal markers that were formed in crosses of the type *P. aeruginosa* PAO × *P. aeruginosa* PAO R, where R is an IncP-1 plasmid. The expectation was that such recombinants would be formed by variants of the IncP-1 plasmid that had acquired the ability to transfer chromosome. A necessary corollary of this argument is that in such recombinants transfer of the plasmid itself would occur in addition to the transfer of chromosome and hence these recombinants would provide a selective system for isolating chromosome mobilizing variants of IncP-1 plasmids.

Experimentally it was found that with R68 it was possible to isolate variants that had acquired highly efficient Cma for strain PAO and other *P. aeruginosa* strains (Holloway, 1974; Haas and Holloway, 1976). The physical examination of R68.45 shows that it has acquired an extra piece of DNA of the order of 1.4–1.6 Mdaltons (Jacob *et al.*, 1977) and there is preliminary evidence that there is an 1800-base pair insertion situated close to the kanamycin resistance marker on the R68 chromosome (van Montagu and Schell, personal communication; Riess, Burkardt and Puhler, personal communication). This last observation is of significance because it has been observed (Haas and Holloway, unpublished observations) that spontaneous loss of the kanamycin marker is usually associated with loss of Cma by R68.45.

This evidence is consistent with the hypothesis that R68.45 has acquired an additional segment of DNA from strain PAO and that this addition confers on R68.45 its unusual Cma properties. Three sites could provide this additional segment of DNA, namely, the *P. aeruginosa* PAO chromosome, resident cryptic plasmids of strain PAO (Pemberton and Clark, 1973), or a duplication of a portion of the R68 genome. Evidence to support the first possibility comes from the fact that crosses of the type PAO × PAO(R68) do not invariably yield recombinants carrying a plasmid of the

R68.45 type. These arise only when particular chromosomal regions are selected; five such regions have been identified (Holloway *et al.*, 1978). This result supports the view that R68.45-like plasmids arise by a transfer of some genetic material from the *P. aeruginosa* chromosome to the R68 plasmid.

The chromosome mobilization abilities of R68.45 are different in one important aspect from that of all other plasmids that can transfer chromosome in *P. aeruginosa*. R68.45 can promote chromosome transfer from many origins and while the number of such origins has not been estimated accurately, seven have certainly been identified and the actual number is likely to be much higher. With R68.45, which was isolated following selection of the *arg*B region (21′) (Haas and Holloway, 1976; Haas *et al.*, 1977), there is no increased frequency for transfer of markers in the *arg*B region, the frequency being the same as that for markers in other regions of the chromosome. Similar properties in terms of the number of origins and the lack of specificity in transferring any particular chromosome region have been shown with R68.45-like plasmids isolated with R68 and other IncP-1 plasmids. They include plasmids isolated by selection for other regions of the PAO chromosome than that used in isolating the original R68.45 (Crowther and Holloway, Nayudu and Holloway, unpublished observations).

There is no direct evidence in *P. aeruginosa* that R68.45 integrates into the chromosome as part of the process of chromosome transfer. Efforts to find Hfr-like derivatives of R68.45 by both selective and nonselective procedures have been entirely unsuccessful (Haas and Holloway, 1976; Haas, personal communication). One unexplained feature of the behavior of R68.45-mediated chromosome transfer is that, in PAO × PAO R68.45 crosses, recombinants for chromosomal markers usually inherit the whole plasmid, but show a marked inability to maintain the plasmid intact.

Stability of IncP-1 plasmids in *P. aeruginosa* is strain dependent (Chandler and Krishnapillai, 1974b). In strain PAO they are highly stable while in strain PAT they are unstable. This instability is not of the type in which the whole plasmid is lost as a unit (hereditary instability). Instead, in PAT, plasmid markers are lost progressively so that clones of strain PAT carrying an IncP-1 plasmid can be isolated in which only a fraction of the identifiable plasmid markers have been retained; with further bacterial replication, more plasmid markers become lost until either the remnant stabilizes as a more or less permanent fraction of the original plasmid (usually Tra^-) or the whole plasmid is lost (Godfrey, Morgan, and Holloway, in preparation). This type of instability will be referred to as structural instability.

Recombinants for bacterial chromosomal markers in PAO × PAO R68.45 crosses show extensive structural instability for plasmid markers but rarely for chromosomal markers (Haas and Holloway, 1976, 1978). After the recombinants had gone through 50 generations, between 40 and 70% of the single clones showed sensitivity to carbenicillin, tetracycline, and kanamycin and had presumably lost the R plasmid. Less than 5% of recombinants retained an entire R68.45 plasmid, but various incomplete R68.45 marker combinations were found. These observations could be interpreted as indicating that, as a stage of mobilization R68.45 is integrated into the chromosome of the donor bacterium, that transfer of chromosome also involves transfer of all or part of the plasmid and that the recombination processes taking place either in the donor or in the recipient parent resulted in deletion of all or part of plasmid genome. This loss of plasmid genome could be due to the production of deletions in genes situated near the locations of insertion sequences (Weisberg and Adhyda, 1977). This structural plasmid instability in recombinants has been observed with R68.45 in *P. aeruginosa* PAO (Haas and Holloway, 1976) and *R. meliloti* (Kondorosi *et al.*, 1977) but not in

R. leguminosarum (Beringer and Hopwood, 1976). A similar structural instability of plasmid markers in chromosomal recombinants has been found with RP4-promoted chromosome mobilization in *Acinetobacter calcoaceticus* (Towner and Vivian, 1976a, b).

A model to explain the properties of R68.45 proposes that R68 has acquired, from the chromosome of strain PAO of *P. aeruginosa*, an additional segment of DNA. The properties of this additional DNA, when present in the R68 genome, include:

(1) the ability to mobilize chromosome from a number of origins in all strains of *P. aeruginosa* tested, including PAO, PAT, PAC, in intra- and interstrain matings of these strains (Haas and Holloway, 1976, 1978); and

(2) the ability to mobilize bacterial chromosome in a range of other bacteria including *P. putida* (Martinez and Clark, 1975; Morgan and Holloway, unpublished data), *P. glycinea* (Fulbright and Leary, unpublished data), *E. coli* (Beringer and Hopwood, 1976; Kondorosi *et al.*, 1977), *R. leguminosarum* (Beringer and Hopwood, 1976; Beringer *et al.*, 1978), *R. meliloti* (Kondorosi *et al.*, 1977), and *Rhodopseudomonas sphaeroides* (Sistrom, 1977). Where the genetic analysis carried out in these various organisms has been sufficiently extensive, it appears that, as with *P. aeruginosa*, R68.45 can promote chromosome transfer from multiple sites on the chromosome. The amount of chromosome transferred in each conjugational event varies. In *P. aeruginosa* PAO, relatively short pieces of donor chromosome are inherited, usually not exceeding the equivalent of 10 min on the current map (Haas and Holloway, 1978). Linkage data from R68.45-mediated crosses in *Rhizobium* suggest that larger fragments are transferred and Kondorosi *et al.* (1977) suggest that up to 30% of the *R. meliloti* chromosome can be recovered.

In view of the involvement of insertion sequences in F integration in *E. coli*, it is reasonable to suggest that insertion sequences could be involved in the mechanism of chromosome mobilization by R68.45. However, at present there is no evidence that insertion sequences occur in *P. aeruginosa*.

The mechanism by which R68.45 mobilizes chromosome is not yet understood. The main facts that must be accounted for are the multiple origins of transfer, the failure to find a stable form in which R68.45 has been integrated into the chromosome, and the ability of R68.45 to mobilize chromosome in such a wide range of genera. The only evidence to support some form of transient or unstable integration of R68.45 as an early stage of chromosome mobilization comes from the structural instability for plasmid markers found in recombinants. Perhaps excision of R68.45 is associated with deletion formation (Weisberg and Adhya, 1977) and such deletions may initiate structural instability, although no molecular mechanisms for this can be suggested at present.

That R68.45 can integrate into the *P. aeruginosa* chromosome is shown by the isolation of R′ derivatives (see below). Stable R′ plasmids have been derived from R68.45 in both *P. aeruginosa* (Holloway, 1978) and *Rhizobium* species (Johnston *et al.*, 1978). However, from what is currently known about the frequency of recovery of stable R′ forms it is not possible to conclude that this is the main mechanism of chromosome transfer. Finally, and particularly in view of the wide bacterial range in which R68.45 has Cma, the possibility of a nonintegrative mechanism of chromosome mobilization cannot be ruled out.

Much remains to be learned about R68.45, including answers to the following questions.

(1) What is the nature of the DNA which has been added to R68 to form R68.45? If this DNA has been acquired from the *P. aeruginosa* PAO chromosome, does this DNA have any special genetic properties in the chromosome, or does it need to be inserted into a plasmid such as R68 for its activity to be displayed?

(2) Can plasmids other than R68 acquire such enhanced Cma?

(3) What is the nature of the relationship between R68.45 and the various bacterial species in which it displays Cma?

(4) Is integration, of any kind, necessary for the Cma of R68.45?

(5) Does *P. aeruginosa* provide a unique source of the DNA segment that, when added to R68, generates R68.45-like plasmids? It would be interesting to see if similar plasmids could be generated from *E. coli* crosses with R68, particularly in view of the extensive knowledge of insertion sequences in that organism.

V. THE FORMATION OF PLASMID CHROMOSOME HYBRIDS

A development contributing to the understanding of how chromosome can be mobilized by plasmids was the isolation of F′ plasmids in *E. coli*. These were shown to be hybrids containing all or most of the F genome plus a segment of the *E. coli* chromosome (Low, 1972; Guyer *et al.*, 1977). They have been particularly useful for studying a variety of problems in microbial genetics including mapping, the mechanisms of chromosome mobilization, and the regulation of protein synthesis in bacteria. F′ plasmids can be isolated by a number of methods, which have been summarized previously (Low, 1972). It has subsequently been shown that similar structures can be formed by other plasmids. Nishimura *et al.* (1973) were able to isolate a plasmid containing the *lac* region of the *E. coli* chromosome integrated into the R plasmid, R100-1. This R′*lac* plasmid was derived by Pl transduction following the integration of the R plasmid into the *E. coli* K12 chromosome through the technique of integrative suppression.

A highly effective technique for the isolation of plasmid chromosome hybrids involves recombination-deficient recipients. In this case the isolation of chromosomal recombinants is dependent upon the transfer of plasmids that have acquired fragments of the bacterial chromosome, the genes of which can be expressed without integration into the recipient chromosome. This technique has been used to isolate a variety of F′ plasmids in *E. coli* (Low, 1968; Ou and Anderson, 1976). More recently, R′ derivatives of R68.45 have been isolated by a similar technique in *P. aeruginosa* (Holloway, 1978). It has been found that the frequency of isolation of these may vary with the region of the chromosome selected. Where selection was made for the *arg*H region, the recovery of R′ structures was of the order of 10^{-8}/donor parent (Holloway, 1978). However, subsequently other regions of *P. aeruginosa* have been used for the selection of R′ plasmids and the frequency of isolation is up to 100 times greater than with the *arg*H region (Royle and Holloway, unpublished data). R′ structures have also been isolated using R68.45 in *R. leguminosarum* by Johnston *et al.* (1978). In this latter case, the R′ plasmids were isolated by crosses between *R. leguminosarum* and *R. meliloti* and could be recovered presumably by the inability of the *R. meliloti* chromosome to be integrated into the *R. leguminosarum* chromosome. R′ plasmids have also been isolated in *R. meliloti* by Kondorosi (personal communication).

The IncP-1 plasmids are particularly effective in forming plasmid-bacterial chromosome hybrids in *Pseudomonas* and *Rhizobium* as described and also in *E. coli* (Olsen and Gonzalez, 1974) and *Klebsiella pneumoniae* (Dixon *et al.*, 1976).

In *P. aeruginosa*, considerable efforts have been made to isolate FP′ structures derived from the various FP plasmids available (Holloway, unpublished observations). FP2 and FP39 have been quite refractory in this respect and the only successful case is that for FP110 which forms FP′ structures at frequencies of ca. 10^{-9}/donor parent in a recombination-deficient recipient. Some, but not all, FP110 hybrid plasmids are structurally stable in hosts with normal

recombinational function (Royle and Holloway, unpublished data).

The finding of plasmid-chromosome hybrid structures which retain all the properties of the plasmid is clear evidence of recombination between two dissimilar DNA sequences and, at least for the F′ plasmids, there is evidence of involvement of particular insertion sequences in the recombination events that lead to their formation (Ohtsubo and Ohtsubo, 1977).

For IncP-1 plasmids such as R68.45, the wide host range combined with the ability to acquire regions of the bacterial chromosome, provides a highly effective means of intergeneric transfer of bacterial genes as well as providing an insight into a mechanism of bacterial evolution that previously has been largely speculative. It has now been shown that R′ plasmids generated in *P. aeruginosa* R68.45 strains can transfer to *E. coli* and there express particular *P. aeruginosa* chromosomal genes including those for tryptophan synthetase (Hedges *et al.*, 1977). Similar plasmids containing other segments of the *P. aeruginosa* chromosome were found to be capable of restoring prototrophy to a variety of *E. coli* auxotrophs (Hedges and Jacob, 1977). Similar studies have been made with *P. aeruginosa* and *P. putida* (Holloway *et al.*, 1978). Domaradskij *et al.* (1976) and Filkova *et al.* (1977) have also studied transfer and expression of *P. aeruginosa* genes in *E. coli* using the IncP-1 plasmid RP1. Indeed in such intergeneric or interspecific crosses, the lack of recombination between the unrelated chromosomes provides the equivalent of the recombination-deficient recipient used in the isolation of F′ or R′ plasmids.

VI. FUNCTION OF THE HOST GENOME IN Cma

There are a variety of responses among different plasmids in different organisms involving aspects of the host genome with respect to Cma. With F in *E. coli*, for example, there are about 24 sites at which F can integrate on the chromosome (Matney *et al.*, 1964) but it is by no means certain that this is the maximum number of origins from which F can mobilize chromosome. In *P. aeruginosa*, most of the plasmids that have been studied show Cma from very few origins. FP2, the plasmid most studied in this respect, has one origin site and the same is true for FP39, FP5, and very likely FP110 (Pemberton and Holloway, 1973; Hughes-Jones and Holloway, unpublished data; Royle and Holloway, unpublished data). With respect to R plasmids in strain PAT, R68 has one origin whereas R91-5 has two, one of which may be the same as the R68 origin (Watson and Holloway, 1978). In *P. aeruginosa* PAO, R68 promotes chromosome transfer at frequencies of less than 10^{-8}/donor parent cell. Very likely this indicates that one chromosomal difference between PAO and PAT is a site at which R68 can mobilize chromosome. As noted, R68.45 has a variety of sites from which it can promote chromosome transfer in both strains PAO and PAT.

In *P. mirabilis*, Coetzee (1978a) has found only one mobilization site with plasmid D, and in this respect, D is similar to R1 with both *E. coli* and *Salmonella typhimurium*. R1-Mediated transfer occurs from a region close to the *trp-cys-pyr* markers although the precise chromosomal site is different for each organism (Pearce and Meynell, 1968; Hedén and Meynell, 1976). This is the same region from which ColV transfers chromosome at high frequency in *E. coli* and the same is true for a group of R factors studied by Hedén and Rutberg (1976) in *E. coli* C.

A further role of the host genome has been revealed by the study of the relationship between plasmid instability and Cma of IncP-1 plasmids in *P. aeruginosa* strain PAT (Holloway *et al.*, 1977; Godfrey, Morgan, and Holloway, in preparation).

As stated above, the plasmid R68 shows highly effective Cma for strain PAT of *P. aeruginosa* but not for strain PAO (Watson and Holloway, 1978) and this is also true of other IncP-1 plasmids. As described above,

IncP-1 plasmids show structural instability in *P. aeruginosa* strain PAT but are highly stable in strain PAO of the same species. In experiments with R68, it has been possible to construct a plasmid chromosome map from the actual frequency of loss of individual markers by what is essentially deletion mapping. This map shows the same order for the markers examined, CB–AR–TC–Cma–KM–Tra, as that found by other workers using different mapping procedures (Stanisich and Bennett, 1976; Barth and Grinter, 1977). The data are in agreement with the view that there is a point of origin for the process leading to loss of markers and the frequency of loss of a particular marker is a function of the distance from that point. This point is close to a region of the plasmid that is responsible for Cma because Cma is the marker most frequently lost. If *P. aeruginosa* PAT R68 is grown for about 18 hs in nutrient broth without selection for any of the antibiotic resistant markers, it is found that, after 25 generations, 24% of the colonies have lost Cma and 8% have lost kanamycin resistance. This correlation between the most frequently lost markers is of interest in relation to the correlation between loss of kanamycin resistance and loss of Cma in R68.45 described above.

This loss of plasmid markers is to some extent a bacterial function because not only is it strain specific, occurring in strain PAT but not PAO, but it is also dependent on a functional *rec*A$^+$ product. With a Rec$^-$ mutant of PAT, R68 is as stable as it is in strain PAO. Furthermore, R68 does not mobilize chromosome in a Rec$^-$ PAT mutant and thus, in these two respects, a Rec$^-$ PAT strain is like strain PAO in its response to R68. Revertants of the Rec function can be obtained and are found to have regained Cma and also to show instability for R68 markers. As pointed out above, chromosomal recombinants from PAO × PAO R68.45 also show high structural instability for plasmid markers. As yet there is no model that can be offered to explain all these results, but it can be concluded that Cma is not entirely a plasmid determined function but also depends upon a host component.

VII. INTERGENERIC MATING

There is considerable current interest in studying the transfer of bacterial genome segments, both plasmid and chromosome, between unrelated bacteria to produce recombinants with new and desired phenotypes, and certain techniques of *in vitro* genetic engineering have been developed for this end. Such genetic exchange *in vivo* has also been described for plasmids in the IncP-1 group (e.g., Jacoby and Shapiro, 1977) and for bacteria in the Enterobacteriaceae (Baron *et al.*, 1968). F can act to promote chromosome transfer in *Escherichia, Shigella,* and *Salmonella* and also in intergeneric crosses between those genera. In the latter cases, the frequency of recovery of recombinants is usually low, but bacterial mutants have been found that have higher recombination frequencies. It is of particular interest that many of the recombinants resulted from the production of plasmid-like structures that did not integrate into the recipient chromosome; some of these partial diploid structures showed unusually high stability. However, *Salmonella* chromosome can be stably inserted into the *E. coli* chromosome. Johnson *et al.* (1975) and Cannon *et al.* (1974) have shown that chromosomal genes of *Klebsiella* concerned with nitrogen fixation can integrate into the *E. coli* chromosome in F-mediated crosses. With one exception to date, the host range of F for chromosome transfer does not extend beyond the Enterobacteria including some plant pathogenic genera such as *Erwinia*. The exception is that F′ plasmids of *E. coli* K12 can be transferred into the strain 6.2 of *Pseudomonas fluorescens* biotype IV. A variety of *P. fluorescens* auxotrophs were complemented by F′ plasmids with chromosomal segments carrying isofunctional genes. The plasmids can be subsequently transferred from *P. fluorescens* to other *P. fluorescens* recipients (Mergeay and Gerits, 1978).

With the demonstration that IncP-1 plas-

mids have both a wide host range and Cma, they are being used increasingly to study exchange of chromosome between unrelated bacteria. The role of R′ plasmids derived from R68.45 in intergeneric matings has been discussed above. Such studies are important both for establishing the nature of the regulatory systems that operate when nonhomologous DNA has the opportunity of producing gene products in a foreign bacterial environment (Hedges *et al.*, 1977; Nagahari *et al.*, 1977) and as an attractive experimental tool for studying bacterial evolution (Reanney, 1976).

It may be possible to select other derivatives of IncP-1 plasmids with special properties of intergeneric transfer of chromosomal segments in much the same way that R68.45 was derived. In the author's experience with such intergeneric transfers with R68.45, it has become clear that care is needed to ensure that the restriction systems of the recipient do not prevent the isolation of the already rare recombinants found in such crosses and also that Cma is still retained by R68.45 after it has been transferred.

VIII. SUMMARY

Cma can now be accepted as a common property of plasmids rather than one limited to a few, intensively studied examples. The mechanisms by which plasmids mobilize chromosome, in particular the role of plasmid integration into the bacterial chromosome and the part played by insertion sequences, are areas to which future interest should be directed. Solutions to these problems will be instructive in our understanding of plasmid and bacterial chromosome structure and function. The role of the IncP-1 plasmids can be expected to be central to this future work.

The wide variety of bacterial genera for which conjugational analysis, mapping, and recombinant isolation are now available has extended microbial genetics into new areas. These advances are promising for the solution of a variety of practical problems in medicine, agriculture, and environmental science in the foreseeable future.

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