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Transduction of Low-Copy Number Plasmids by Bacteriophage P22

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ABSTRACT

The generalized transducing bacteriophage of *Salmonella typhimurium*, P22, can transduce plasmids in addition to chromosomal markers. Previous studies have concentrated on transduction of pBR322 by P22 and P22HT, the high transducing mutant of P22. This study investigates the mechanism of P22HT transduction of low-copy number plasmids, namely pSC101 derivatives. We show that P22HT transduces pSC101 derivatives that share homology with the chromosome by two distinct mechanisms. In the first mechanism, the plasmid integrates into the chromosome of the donor by homologous recombination. This chromosomal fragment is then packaged in the transducing particle. The second mechanism is a size-dependent mechanism involving a putative plasmid multimer. We propose that this multimer is formed by interplasmidic recombination. In contrast, P22HT can efficiently transduce pBR322 by a third mechanism, which is independent of plasmid homology with the chromosome. It has been proposed that the phage packages a linear concatemer created during rolling circle replication of pBR322, similar in fashion to phage genome packaging. This study investigates the role of RecA, RecD, and RecF recombination proteins in plasmid/plasmid and plasmid/chromosome interactions that form packageable substrates in the donor. We also examine the resolution of various transduced plasmid species in the recipient and the roles of RecA and RecD in these processes.

THE temperate bacteriophage, P22, mediates generalized transduction in *Salmonella typhimurium* (ZINDER and LEDERBERG 1952). Wild-type P22 and mutant derivatives are valuable and versatile tools used in Salmonella genetics. P22 packages its genome starting at a *pac* site on a linear multimer created by rolling circle replication (TYE *et al.* 1974; reviewed in POTEETE 1988). The linear phage DNA is cut into 44-kb segments, referred to as a headful, the minimum amount of DNA required for packaging (TYE *et al.* 1974). It has been shown that P22 can also mediate plasmid transduction, or the movement of nonchromosomal DNA from one cell to another (ORBACH and JACKSON 1982; SANDERSON and ROTH 1988; GARZON *et al.* 1995). In studies using wild-type P22, a concatemer of plasmid pBR322 could be packaged when the vector contained a P22 *pac* site or a *pac* analogue from the bacterial chromosome (SCHMIEGER 1982; SCHMIDT and SCHMIEGER 1984; VOGEL and SCHMIEGER 1986). The high transducing mutant of P22, P22HT, can transduce pBR322 independent of a wild-type *pac* site because the phage apparently has reduced stringency for *pac* recognition (MARGOLIN 1987). It has been proposed that P22 infection allows for productive rolling circle replication of pBR322 and this provides a substrate for headful packaging into the phage head (Figure 1C). Indeed, this phenomenon of plasmid transduction has been seen in several other systems in both gram negative and gram positive species (VIRET *et al.* 1991).

In contrast to pBR322, low-copy number plasmids are not efficiently transduced. Previous evidence suggested that these plasmids required homologous recombination with the host chromosome to be packaged by a bacteriophage. For example, TRUN and SILHAVY showed that a pSC101 derivative containing homology with the *Escherichia coli* chromosome could be transduced by bacteriophage P1 (TRUN and SILHAVY 1987). Apparently, the phage packages a chromosomal fragment that contains a plasmid that has integrated into the chromosome by homologous recombination. This integrated plasmid recircularizes in the recipient. Thus, phage packaging essentially traps the intermediate of a double recombination event between the plasmid and the bacterial chromosome (Figure 1A). Indeed, these authors used this recombination-dependent transduction to map mutations within the cloned chromosomal fragment. We have previously provided similar results showing that transduction of the R6K derivative pGP704 by P22HT*int* was dependent on the plasmid containing a cloned chromosomal fragment. Again, the phage apparently packages a chromosomal fragment containing an integrated plasmid that resulted from homologous recombination with the chromosome (MAHAN *et al.* 1993).

Induction of rolling circle replication of pBR322 has been seen in a variety of conditions. A common prerequisite in these cases is the inhibition of the RecBCD exonuclease activity that would normally prevent the

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accumulation of linear concatemeric DNA. This can be accomplished in the appropriate mutant background (COHEN and CLARK 1986; SILBERSTEIN and COHEN 1987) or by using phage encoded functions that inhibit RecBCD to allow rolling circle replication of the phage genome (COHEN and CLARK 1986; POTEETE *et al.* 1988). RecA has also been implicated in pBR322 rolling circle replication (COHEN and CLARK 1986). Plasmid concatemers can also be generated by interplasmidic recombination. RecF has been implicated in both interplasmidic and intraplasmidic recombination in otherwise wild-type cells (COHEN and LABAN 1983; KOLODNER *et al.* 1985).

If low-copy number plasmids require homologous recombination with the chromosome to be transduced, this provides a mechanism to facilitate allelic exchange between plasmid and chromosomal sequences. To determine the feasibility of this technique, we examined the mechanism of low-copy number plasmid transduction in more detail. In this study, we show that a pSC101 derivative is transduced by bacteriophage P22HT*int* by two distinct mechanisms (Figure 1). The first mechanism requires integration of the plasmid into the donor chromosome by homologous recombination. The second mechanism is independent of chromosomal homology but is apparently distinct from the mechanism involved in the transduction of pBR322. We also examine the role of various recombination proteins in the generation of packageable substrates in the donor and the resolution of plasmids in the recipient.

MATERIALS AND METHODS

Bacterial strains and plasmids: The bacterial strains used in this study are listed in Table 1. All strains are isogenic derivatives of the virulent *S. typhimurium* strain ATCC 14028 (CDC 6516-60).

Media and chemicals: Growth media were prepared as described (DAVIS *et al.* 1980). Bacteriophage were diluted in T2 buffer (MALOY *et al.* 1996). Antibiotics were used at the following concentrations: Ampicillin (Ap), 50 μg/ml; Kanamycin (Kn), 50 μg/ml; Spectinomycin (Sp), 125 μg/ml; Tetracycline (Tc), 25 μg/ml. Replica plates used to detect exchange of markers contained Ampicillin 100 μg/ml and Kanamycin 50 μg/ml, except for the *recF* donor experiments where the plates contained Ampicillin 50 μg/ml and Spectinomycin 125 μg/ml. All replica plates used in testing transductants contained 10 mM EGTA.

Transduction methods: Bacteriophage P22 lysates were prepared according to DAVIS *et al.* (1980). Phage were titered (DAVIS *et al.* 1980) using T2 buffer. For transductions of JS111, 0.1 ml of a 1:100 dilution of phage and 0.1 ml of an overnight culture of JS111 were mixed with 1 ml of LB and incubated at 37° for 30 min. The mixture was centrifuged, the supernatant was removed and the bacterial pellet was resuspended in 1 ml of 150 mM NaCl. The transduction mixture was centrifuged again, the supernatant was removed, and the pellet was resuspended in 210 μl of 150 mM NaCl. The washing procedure was carried out to reduce nutrient transfer to the minimal plate. Aliquots of 100 μl were plated on LB containing Ampicillin and on E minimal glucose agar to select for Ap^r transductants and Pyr^+ transductants, respectively. This procedure allowed us to directly compare transduction of the plasmid (Ap^r) to transduction of a chromosomal marker (Pyr^+) to determine the efficiency of plasmid transduction (EOPT). Transductions into the *recA, recD,* and control recipients were performed in an analogous fashion except that they were plated on LB Ap and LB Kn to select for transduction of the plasmid and the chromosomal marker, respectively.

Determination of the frequency that a transduced marker recombines with the recipient chromosome: Transduction of plasmid pJS225 from strain JS107 results in 12% of the Ap^r transductants being Kn^r. The inheritance of the Kn^r marker could be the result of the plasmid recircularizing with the Kn^r marker on the plasmid or, alternatively, the plasmid could recircularize without the Kn^r marker and the remaining transduced fragment could recombine with the recipient chromosome. To determine the frequency of this second event, we chose 145 Ap^r Kn^r plasmid transductants, which were subsequently streaked free of phage. The 145 samples were then grouped into 29 sets of five each. Each set was grown in L broth with Ap. Alkaline lysis plasmid preparations (AUSUBEL *et al.* 1987) were done on all 29 sets and DNA from each was electroporated into strain SH6749 and diluted and plated on LB Ap plates. The appropriate dilution plate was then replica plated onto LB Kn Ap agar. If one of the five transductants in a set carried the Kn marker in the chromosome rather than the plasmid, then ~20% of the Ap^r transformants should be Kn^s. If a set gave Kn^s colonies, then the five samples in the group were grown separately and used to make P22HT*int* lysates. The five lysates were then used to transduce JS112 (*purA*::Sp), selecting for Pur+. The *purA*$^+$ allele is tightly linked to the Kn marker and is carried both on the plasmid pJS225 and in the chromosome of the donor, both of which can be trasnduced into the recipient. The Pur^+ transductants were scored for Kn^r. Eight Kn^r and eight Kn^s colonies from each transduction were independently scored for Ap^r and Sp^r. If a lysate gave Pur^+ Kn^s Sp^r Ap^r colonies (transduction of a *purA*$^+$ Kn^s plasmid) and Pur^+ Kn^r Sp^s Ap^s colonies (transduction of the Kn cassette from the chromosome), then we concluded that this sample has the Kn^r cassette in the chromosome.

Determination of plasmid copy number in *S. typhimurium*: The pSC101 derivative plasmid pJS225 and the pBR322 derivative plasmid pBAM362 were independently transformed into JS83. These plasmids contain the identical chromosomal insert. Total cellular DNA was isolated from overnight L Ap broth cultures as described (MALOY *et al.* 1996). A *Cla*I restriction enzyme digest was done on all samples, giving linear plasmids. *Cla*I cuts outside the region of homology in both plasmids. Three independent samples of each plasmid were separated on a 0.8% SeaKem agarose gel. The DNA was transferred to a nitrocellulose membrane and the membrane was probed with a ^{32}P α-dCTP labeled probe using a standard Southern Blot protocol (AUSUBEL *et al.* 1987). The probe used is an internal *Pst*I fragment of the region of homology shared by both plasmids. Thus the region of DNA that hybridizes to the probe is identical in both plasmids and in the chromosome. The membrane was viewed using a Molecular Dynamics Phosphorimager and the relative intensity of the bands was determined using the Image Quant software. We compared the intensity of the plasmid band *vs.* the chromosomal band in each lane. Defining the chromosomal band as 1.0 for stationary phase cells, we then determined the average number of copies of a plasmid in any particular sample.

RESULTS

P22HT*int* transduces pSC101 derivatives and pBR322 by distinct mechanisms: To test the mecha-

TABLE 1

Bacterial strains and plasmids

Salmonella strains/ plasmids/phage	Genotype	Origin/reference[a]
A. Bacteria		
14028	Wild type	ATCC
MT96	14028 *oafA126*::Tn*d*Tc	SLAUCH *et al.* (1995)
JS83	14028 *galE496*	
JS106	14028 *recD542*::Tn10*d*Cm	
JS107	14028 *zjg8101*::Kn	
JS108	14028 *zjg8101*::Kn *recD542*::Tn10*d*Cm	
JS109	14028 *recF522*::Tn5	
JS110	14028 *recA1*	
JS111	14028 *pyrC691*::Tn10	
JS112	14028 *purA*::Sp	
SH6749	LT2 *hisC527 ilv-452 metA22 metE551 trpB2 galE496 xyl404 rpsL120 flaA66 hsdL6 hsdSA29*	HARKKI and PALVA (1984)
B. Plasmids		
pWKS30	pSC101 ori, Ap^r	WANG and KUSHNER (1991)
pJS225	pWKS30, 5-kb 14028 DNA insert[b]	
pJS226	pJS225, Sp^r cassette in insert	
pBAM26	pJS225, Kn^r cassette in insert	
pJS204	pWKS30, 5-kb 14028 DNA insert[c]	
pAL205	pJS204::Mu*dJ*	
pAL215	pJS204::Mu*dJ*[d]	
pBAM31	pWKS30::Mu*dJ*	
pBAM32	pWKS30, Tc^r 1.3-kb from pBR322	
pBR322	ColE1 ori, Ap^r	Lab stock
pBAM326	ColE1 ori, Ap^r 5-kb 14028 DNA[b]	
pBAM362	ColE1 ori, Ap^r 5-kb 14028 DNA[e]	
C. Bacteriophage		
P22HT*int*	HT105/1 *int-201*	SCHMIEGER (1972)

[a] This study if not otherwise noted.
[b] This 5-kb region of 14028 DNA is at *purA*.
[c] This 5-kb region of 14028 DNA is at *oafA*.
[d] The Mu*dJ* insert in pAL215 is distinct from the one in pAL205.
[e] The 5-kb region of 14028 DNA in pBAM362 is in the opposite orientation as the region in pBAM326.

nism of low-copy number plasmid transduction, we used a pSC101 derivative, pWKS30 (WANG and KUSHNER 1991), which has no known homology with the Salmonella chromosome. We cloned a 5-kb fragment of *S. typhimurium* chromosome into pWKS30, yielding plasmid pJS225. Both pWKS30 and pJS225 were electroporated into JS107, a derivative of *S. typhimurium* strain 14028 that contains a Kanamycin-resistance cassette in the middle of the region of chromosomal homology shared with pJS225. This insertion mutation confers no apparent phenotype to the cell other than Kn^r. We also cloned a different 5-kb fragment of *S. typhimurium* chromosome into pWKS30 to make the plasmid pJS204. In this case, plasmid pJS204 is in the strain MT96, which has a Tn*10d*Tc insertion marker in the middle of the region of homology shared with the plasmid. P22HT*int* lysates were grown on each strain and used to transduce strain JS111, a *pyrC* auxotrophic derivative of strain 14028. Equal volumes of the transduction mixture were plated on LB containing ampicillin and on glucose minimal medium to select for Ap^r transductants and Pyr^+ transductants, respectively. This method allows us to directly compare transduction of the plasmid (Ap^r) to transduction of a chromosomal marker (*pyrC*$^+$) to determine the efficiency of plasmid transduction (EOPT). Thus, each transduction is internally controlled for any possible growth differences in the donor or recipient strain or fluctuations in phage titers. The results in Table 2 indicate that the plasmid without homology, pWKS30, was transduced with an efficiency 8% of that seen for the chromosomal marker. The plasmids containing homology, pJS225 and pJS204, were transduced at 125% and 64% of the efficiency of the chromosomal marker, respectively. These results were consistent with the hypothesis that the transduction efficiency of pWKS30 derivatives was stimulated by homology to the chromosome.

As a control, a P22HT*int* lysate grown on wild-type strain 14028 containing pBR322 was used to transduce JS111 (*pyrC*) as described above. The results show that,

TABLE 2

Plasmid transduction from a wild-type donor

Plasmid	Size (kb)	EPOT (%)[a]	Exchange of marker (%)
pWKS30	5.4	7.9 ± 2.5	<0.69
pJS225[b]	10.4	125.3 ± 90.0	11.9
pBAM26[b]	10.7	111.0 ± 58.0	4.9
pBAM31	15.4	329.2 ± 229.3	NA
pJS204[b]	10.4	63.6 ± 19.7	6.0
pAL205[b]	19.4	562.7 ± 151.7	1.1
pAL215[b]	19.4	558.6 ± 190.6	1.4
pBAM32	6.7	18.0 ± 8.7	NA
pBR322	4.3	310.6 ± 51.3	NA
pBAM326[b]	9.3	1044 ± 932	0.045
pBAM362[b]	9.3	2358 ± 1121	0.45

[a] EOPT is the efficiency of plasmid transduction and is given as average ± SD where $n \geq 7$. NA, not applicable.
[b] Plasmids that share homology with the chromosome.

consistent with previous results (SANDERSON and ROTH 1988), plasmid pBR322 is transduced threefold more efficiently than a chromosomal marker. Note that the plasmids pBR322 and pWKS30 are similar in size and neither shares any known homology with the Salmonella chromosome. NOVICK *et al.* (1986) showed that the frequency of transduction of plasmid concatemers generated by rolling circle replication was directly proportional to plasmid copy number using phage $\phi 11$ in Staphylococcus. However, copy number does not explain the difference in efficiency of plasmid transduction in our studies. We determined the copy number of pSC101 derivatives to be 7.86 ± 0.54 and the copy number of pBR322 derivatives to be 13.93 ± 2.0. Clearly a twofold difference in copy number cannot account for such a drastic difference in EOPT. Taken together, these results suggest that the pSC101 derivatives and pBR322 are transduced by distinct mechanisms.

Derivatives of plasmid pWKS30 that share homology with the chromosome are transduced by two distinct mechanisms: If transduction of pJS225 is solely dependent on integration of the plasmid into the bacterial chromosome, then there should be significant genetic linkage between the Ap^r marker on the plasmid and the Kn^r cassette in the chromosome. Presumably, when the integrated plasmid pJS225 is transduced, the transducing particle contains a duplication of 5 kb with one copy of the duplicated region containing the Kn^r cassette. These two regions of homology recombine during plasmid recircularization in the recipient. Since the Kn^r cassette is in the middle of the homologous region, 50% of the circularized plasmids should contain the Kn^r cassette. We scored 1200 pJS225 Ap^r transductants for Kn^r to test the frequency that the plasmid is transduced as a chromosomal integrant. It was shown that the Kn^r cassette was linked to the plasmid in only 12% of the clones (Table 2). Similar results were obtained for plasmid pJS204 in that the Tn*10d*Tc insertion marker was linked to the plasmid in only 6% of the transductants. This suggests that plasmids pJS225 and pJS204 are not transduced as integrants as often as expected and that some additional transduction mechanism may be involved. This second mechanism may explain how plasmid pWKS30, which has no homology with the chromosome, is transduced at low frequency.

The apparent low frequency of plasmid recombination with the chromosome during a transduction could result from a selective disadvantage of having the insertion on the plasmid such that plasmids without the insertion are recovered preferentially. We tested this directly by exchanging the alleles between the plasmid pJS225 and the donor chromosome. If there is a selection against recircularization of the plasmid with the Kn^r marker, then the same result should be obtained whether the Kn^r marker is originally in the chromosome or on the plasmid in the donor strain. The plasmid pBAM26 is identical to pJS225 except that it contains the Kn^r cassette in the middle of the cloned chromosomal fragment. A P22HT*int* lysate grown on wild-type strain 14028 carrying pBAM26 was used to transduce JS111 (*pyrC*). As expected, this plasmid was transduced equally as well as the chromosomal *pyrC* marker (Table 2). However, 95% of the Ap^r transductants were also Kn^r. In other words, the plasmid exchanged the marker with the chromosome only 5% of the time. These results are consistent with the previous interpretation that the plasmid is being transduced by two separate mechanisms. A proportion of the plasmids are transduced via integration into the donor chromosome. Because the Kn^r plasmids represent 50% of the integrants, we conclude that 10–25% of the plasmids are transduced as integrants. The majority of the plasmids are transduced by a mechanism that does not involve interaction with the donor chromosome. The fact that similar results are obtained with plasmids pJS225 and pJS204 suggests that this phenomenon is not the result of some specific sequence cloned into plasmid pWKS30. Since the phage packages ~44 kb of DNA, we presume that this second mechanism involves transduction of a plasmid multimer (Figure 1B), as has been observed in a variety of systems (VIRET *et al.* 1991).

It is formally possible that the Kn^r plasmid transductants of pJS225 are not the result of transducing an integrated plasmid, but rather that the plasmid underwent a double recombination event in the donor and thereby obtained the chromosomal Kn^r marker. Subsequently, the plasmid could be transduced by a mechanism that does not require recombination with the chromosome. We tested the frequency at which the plasmid undergoes this double recombination event by determining the frequency of Kn^r plasmids in the population. Only 1 in 12,000 plasmids isolated from an uninfected cell carried the Kn^r marker. These results suggest that the 12% linkage between transduction of Ap^r and

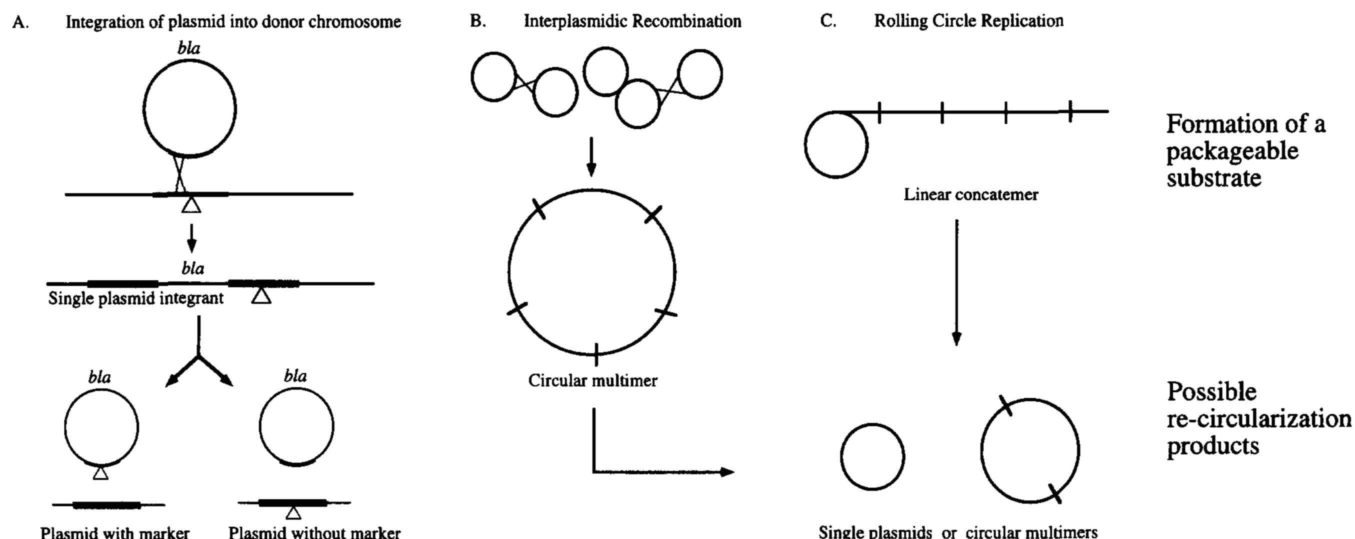

FIGURE 1.—Mechanisms for the formation of packageable substrates and resolution of plasmids in the recipient. (A) A plasmid that contains homology to the chromosome can integrate via homologous recombination and thus be packaged in a 44-kb chromosomal fragment by P22HT. The transduced integrated plasmid can recircularize in the recipient to give two possible plasmid species with respect to a chromosomal marker from the donor. The resulting transducing fragments may subsequently interact with the recipient chromosome. (B) Multiple copies of plasmid can undergo interplasmidic recombination to provide a 44-kb segment of DNA to be packaged. (C) pBR322 can provide a 44-kb segment of DNA to be packaged by undergoing productive rolling circle replication. Transduced plasmid multimers (B and C) can be resolved in the recipient as single copies of free plasmid or possibly circular multimers.

Kn^r is indeed the result of transducing the integrated plasmid.

Transduction of pSC101 derivatives is size dependent: If pJS225 and pJS204 are being transduced by a mechanism that does not involve homologous recombination with the donor chromosome, then why are these plasmids transduced more efficiently than pWKS30? One possibility is that transduction by the alternative mechanism is size dependent. To test this hypothesis, we created a large pWKS30 derivative with no known homology with the Salmonella chromosome. This plasmid, pBAM31, was made by transposing a Mu*dJ* element (CASTILHO *et al.* 1984) onto pWKS30, in effect tripling its size. This larger plasmid was transduced (into strain JS111) threefold better than the chromosomal *pyrC* marker and 42-fold better than the smaller parental plasmid (Table 2). A second test was done using two other plasmids, pAL205 and pAL215. These plasmids contain two distinct Mu*dJ* insertions in the cloned *S. typhimurium* DNA in pJS204. These two plasmids are over 19 kb in size and are transduced 5.5-fold more efficiently than the chromosomal marker (Table 2). These results suggest that the second mechanism of transduction of the pSC101 derivatives is size dependent. This size dependence of transduction of pWKS30 derivatives is evident in Figure 2, where the efficiency of plasmid transduction is plotted *vs.* the size of the pWKS30 derivative.

Transduction of pSC101 derivatives is apparently independent of sequence: It is possible that the larger pSC101 plasmids are transduced more efficiently because of the presence of a particular sequence in these plasmids. For example, these larger plasmids could have more or better P22HT *pac* sites and thus be recognized and packaged more efficiently by P22HT. This packaging efficiency model could account for the extremely efficient transduction of pBR322. CASJENS *et al.* (1987) have defined a 12-bp consensus P22HT *pac* sequence. We searched for this consensus *pac* sequence in pBR322 and found that the two best matches in the sequence correspond to the 3′ end of the *tet* gene. To test the hypothesis that this sequence is responsible for efficient pBR322 transduction, we cloned the *tet* gene from pBR322 into pWKS30 to make pBAM32. A P22HT*int* lysate on wild-type 14028 containing pBAM32 was used to transduce JS111 (*pyrC*). The results (Table 2) show that pBAM32 is transduced only slightly better than pWKS30. Indeed, the transduction efficiency of pBAM32 falls on the curve plotted in Figure 2. Although this experiment was based only on our limited knowledge of *pac* site recognition, it is consistent with the idea that plasmid size is more important than absolute sequence.

Transduction of pBR322: Transduction of pBR322 by P22HT*int* is very efficient even though this plasmid is only 4.3 kb (approximately the same size as pWKS30) and does not contain homology with the chromosome. We tested the effect of adding to pBR322 the 5-kb region of homology that pJS225 shares with the *S. typhimurium* chromosome. In this experiment we can also determine the relative frequency with which this pBR322 derivative is transduced as an integrant in the donor

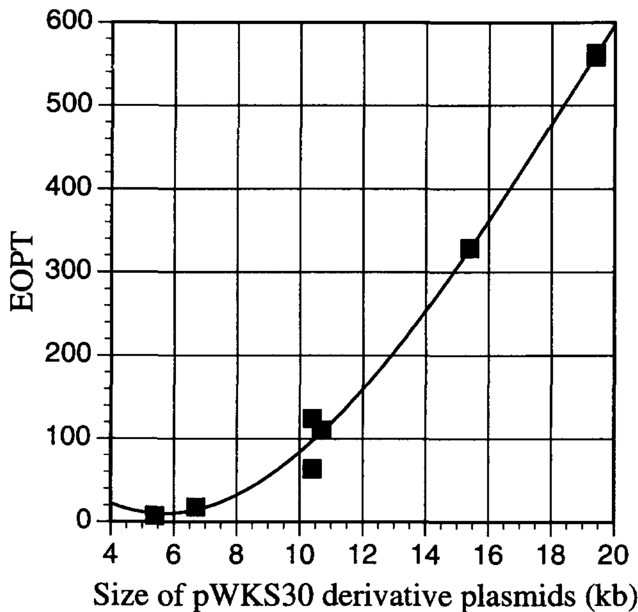

FIGURE 2.—Plasmid transduction is size dependent. Efficiency of plasmid transduction (EOPT) is plotted *vs.* plasmid size for pWKS30 derivative plasmids. Data are from Table 2.

chromosome by determining the linkage to the chromosomal Kn^r marker. The 5-kb region of homology from plasmid pJS225 was cloned into pBR322, giving two plasmids, each having the fragment in a different orientation as determined by restriction analysis. The two plasmids, pBAM326 and pBAM362, were introduced into strain JS107 (*zjg8101*::Kn) and P22HT*int* lysates were made and used to transduce recipient strain JS111 (*pyrC*). The EOPT of each plasmid (Table 2) was increased dramatically over the EOPT of parental plasmid pBR322. Indeed, the effect of increased size on transduction efficiency is much more drastic than that seen with pWKS30 derivatives. Moreover, whereas plasmid pJS225 recombines with the Kn^r marker from the chromosome 12% of the time, plasmids pBAM326 and pBAM362 recombine with the chromosomal marker only 0.045% and 0.45% of the time, respectively. These results reiterate the mechanistic difference between transduction of pBR322 and pWKS30 derivatives.

The plasmid pJS225 (derived from pWKS30), and plasmids pBAM326 and pBAM362 (derived from pBR322) share the same amount of sequence homology with the chromosome. Indeed, when the absolute number of Ap^r transductants that are also Kn^r is compared to the transduction of the *pyrC* marker, it is evident that the number of plasmids that are transduced as an integrant in the donor chromosome is approximately equal in each case (data from Table 2). In addition, we have determined the frequency with which each plasmid interacts with the chromosome by performing transductions where we select for the Kn^r chromosomal marker and then score for coinheritance of the plasmid. In other words, at what frequency is the plasmid integrated into the donor chromosome at the time the chromosomal marker is packaged. In all three cases, this frequency was 3–6.7%. Thus, all three plasmids can be transduced as an integrant. However, the pBR322 plasmids are apparently transduced by an additional mechanism that is not available to the pWKS30 derivatives.

TABLE 3

Plasmid transduction from a *recA* donor

Plasmid	EOPT (%)[a]	Exchange of marker (%)
pWKS30	2.1 ± 1.6	NA
pBAM26[b]	9.6 ± 3.3	<0.33
pBAM31	37.8 ± 8.9	NA
pBR322	36.5 ± 9.5	NA

[a] EOPT is the efficiency of plasmid transduction and is given as average ± SD where $n \geq 4$. NA, not applicable.
[b] Plasmids that share homology with the chromosome.

Formation of packageable substrates in the donor: The data presented above suggests that pWKS30 is transduced predominantly by a mechanism that does not involve interaction with the chromosome and that this mechanism is different than that responsible for the majority of transduction of pBR322. It seems plausible that transduction of both plasmids requires packaging of a concatemer or multimer (SCHMIDT and SCHMIEGER 1984; POTEETE 1988). To understand the formation of the packageable species, we examined the role of various recombination proteins in the donor strain on transduction of pSC101 derivatives. We first tested the role of RecA in plasmid transduction. Four plasmids (pWKS30, pBAM26, pBAM31 and pBR322) were electroporated into JS110, a *recA1* mutant of strain 14028, and P22HT*int* lysates grown on the four strains were used to transduce a *recA*$^+$ recipient strain, JS111 (*pyrC*). The transduction efficiency of all plasmids tested, including pBR322, was reduced five- to 10-fold by the *recA* mutation (Table 3). Thus RecA is involved in, but not absolutely required for, the transduction of both pBR322 and pSC101 derivatives. Although the pSC101 derivative plasmids were still transducable from the *recA* strain, integration of the plasmids containing homology into the donor chromosome was eliminated. The pWSK30 derivative plasmid pBAM26 contains the Kn^r marker in the middle of the 5-kb region of chromosomal homology. None (<0.33%) of the over 300 Ap^r transductants of pBAM26 from the *recA* donor showed loss of the Kn^r cassette compared to 4.9% from the wild-type donor.

The *recF* pathway of recombination has been shown to affect intermolecular plasmidic recombination (COHEN and LABAN 1983; KOLODNER *et al.* 1985). Because this is a potential source of multimeric plasmid, we tested the role of RecF in the donor strain on plasmid transduction. A *recF*::Tn*5* strain, JS109, was constructed and

TABLE 4

Plasmid transduction from a *recF* donor

Plasmid	EOPT (%)[a]	Exchange of marker (%)
pWKS30	2.9 ± 1.5	NA
pJS226[b]	43.9 ± 24.5	2.3
pBR322	284.5 ± 61.9	NA

[a] EOPT is the efficiency of plasmid transduction and is given as average ± SD where $n \geq 10$. NA, not applicable.
[b] Plasmids that share homology with the chromosome.

TABLE 5

Plasmid transduction from a *recD* donor

Plasmid	EOPT (%)[a]	Exchange of marker (%)
pWKS30	86 ± 17	NA
pJS225[b]	1010 ± 174	10.0
pBR322	753 ± 191	NA

[a] EOPT is the efficiency of plasmid transduction and is given as average ± SD where $n \geq 5$. NA, not applicable.
[b] Plasmids that share homology with the chromosome.

electroporated with three plasmids: pWKS30, pBR322, and the pWKS30 derivative plasmid pJS226. The plasmid pJS226 contains the 5-kb fragment of chromosomal DNA with a Sp^r cassette (1.9 kb) cloned into the unique *Eco*RI site in place of the Kn^r cassette (1.3 kb) used in previous experiments. P22HT*int* lysates were grown on each of the strains and used to transduce the *rec*$^+$ recipient strain JS111 (*pyrC*). The transduction efficiency of pWKS30 from the *recF* donor was 2.7-fold lower than seen for the *recF*$^+$ donor but the transduction efficiency of pBR322 was not significantly affected (Table 4). This result shows that the transduction of the low-copy number plasmid pWKS30 is somewhat dependent on RecF in the donor while transduction of pBR322 is independent of RecF.

Multiple lysates of pJS226 in the recF donor JS109 were used to transduce the *rec*$^+$ recipient strain JS111. Analogous to the parental plasmid pWKS30, the transduction efficiency of this plasmid was reduced an average of 2.5-fold by the donor *recF* mutation, with one lysate consistently giving a transduction efficiency reduced 6.5-fold compared to the *recF*$^+$ donor. The plasmid pJS226 can still interact with the chromosome of the *recF* strain and be transduced as an integrant. Scoring over 300 Ap^r transductants for Sp^r showed that plasmid pJS226 lost the Sp^r cassette at a frequency of 2%.

The RecBCD complex is an exonuclease that digests linear DNA at double-stranded breaks (reviewed in Kowalczykowski *et al.* 1994). It has been hypothesized that pBR322 is packaged by P22 during rolling circle replication that produces a linear plasmid concatemer with a double-stranded end (Cohen and Clark 1986; Silberstein and Cohen 1987). We tested the effect of a donor *recD* mutation on transduction of the various plasmids. We electroporated pWKS30, pJS225, and pBR322 into the *recD* donor, JS108, and P22HT*int* lysates were made and used to transduce JS111 (*pyrC*). The results (Table 5) showed that the *recD* mutation caused an approximately 10-fold increase in the EOPT of pWKS30 derivatives while the EOPT of pBR322 increased only threefold. Interestingly, the proportion of the pWKS30 derivative plasmid pJS225 transduced as an integrant in the chromosome is approximately the same as that seen in the *rec*$^+$ donor. These results suggest that the exonuclease activity of the RecBCD complex normally inhibits the formation of packeagable substrates for the pWSK30 derivatives. It follows that both mechanisms of pWKS30 derivative plasmid transduction involve a double-stranded end.

Interaction of the transduced fragment with the recipient chromosome: When the pWSK30 derivative pJS225 is transduced from the donor JS107, which contains a Kn^r cassette in the middle of the homology shared with the plasmid, 12% of the Ap^r transductants are also Kn^r, apparently as a result of transducing a chromosomal fragment that contains an integrated plasmid. Thus, the inheritance of the Kn^r marker could be the result of the plasmid recircularizing with the Kn^r marker on the plasmid or, alternatively, the plasmid could recircularize without the Kn^r marker and the remaining transduced fragment could recombine with the recipient chromosome. To determine the frequency of this second event, we measured the percentage of Ap^r, Kn^r transductants that contained the Kn^r marker in the chromosome of the recipient. Of 145 Ap^r Kn^r transductants, 13 had the Kn^r marker in the chromosome. Assuming that this represents 50% of the events, ~18% of the transduced particles that contain an integrated plasmid undergo a recombination event with the recipient chromosome. This is consistent with previous results for the frequency of interaction of a transducing particle with the chromosome (Ebel-Tsipis *et al.* 1972) and provides further evidence that the genetic linkage between the Kn^r and Ap^r markers is the result of transduction of the integrated plasmid.

Recircularization of the plasmid in the recipient: Presumably, recircularization of the plasmid in the recipient is mediated by homologous recombination between duplicated regions (Orbach and Jackson 1982). To test if the recircularization of the pSC101 derivative plasmids was RecA dependent, the plasmid pJS225 was transduced from donor strain JS107 (contains the Kn^r marker in the chromosome) into isogenic *recA* and wild-type 14028 strains at a MOI of 0.2. All transduction mixtures were divided and plated on LB Ap and LB Kn to independently select for transduction of the plasmid and the chromosomal marker, respectively. Note that the chromosomal marker (Kn^r) can be transduced either by itself or in association with the plasmid. The data (Table 6) shows that the plasmid can recircularize

TABLE 6

Transduction of plasmid pJS225 into *rec* recipients

Recipient	Ap^r transductants		Kn^r transductants	
	Number[a]	% Kn^r	Number[a]	% Ap^r
Wild type	231	14	582	6
recA	48.1	3	0.8	100
Wild type	87	16.5	372	4.3
recD	133.3	19	1019	2.9

[a] The number of transductants is an average per transduction where $N = 3$ for the wild-type recipient and for the *recD* recipient, while $N = 10$ for the *recA* recipient. For the top two lines transductions were done at an MOI of 0.2, while for the bottom two lines transductions were done at an MOI of 0.04.

in the *recA* recipient although the frequency is down slightly. Moreover, the effect of *recA* differentiates the plasmids transduced as an integrant *vs.* those transduced as a putative concatemer, in that the percentage of Kn^r transductants is decreased from 14% to 3%. Thus, the putative concatemer is more efficiently recircularized than the integrant in the *recA* recipient. As expected, recombination into the *recA* recipient chromosome was negligible; transduction of the chromosomal Kn^r marker from the donor was completely dependent on transduction of the plasmid.

Finally, we examined what effect a recipient *recD* mutation had on transduction of pWKS30 derivatives. The plasmid pJS225 was transduced from donor strain JS107 (contains the Kn^r marker in the chromosome) into isogenic *recD* (JS106) and wild-type 10428 at an MOI of 0.04 selecting for plasmid transduction (Ap^r) or chromosomal transduction (Kn^r). Table 6 shows that transduction of the plasmid is increased slightly in the *recD* recipient whereas transduction of the chromosomal marker is increased threefold. The relative frequency of transducing the putative multimer and an integrated plasmid is similar to that seen in a rec^+ recipient.

DISCUSSION

The high-transducing mutant of bacteriophage P22 is capable of carrying out generalized transduction of plasmids in *S. typhimurium.* For *colE1* plasmids, such as pBR322, it has been proposed that productive rolling circle replication of the plasmid in the donor allows packaging of the concatemeric DNA molecule by P22 (Schmidt and Schmieger 1984). Low-copy plasmids, such as pSC101 derivatives, are also transduced by P22HT. We provide evidence that this transduction takes place by two separate mechanisms, both of which differ from transduction of pBR322 (Figure 1).

The first mechanism of pSC101 transduction involves those plasmids that have homology with the bacterial chromosome (Figure 1A). In these cases, the phage packages a chromosomal fragment containing a plasmid integrated by homologous recombination. This structure contains the plasmid sequences flanked by direct repeats of chromosomal DNA. We have observed these events by using a donor that has an antibiotic-resistance marker in the middle of the region of homology shared by the plasmid and the chromosome. In the recipient, this structure resolves itself yielding the plasmid and the remaining chromosomal transducing fragment. Approximately 18% of the remaining transducing fragments recombine with the recipient chromosome.

The second mechanism of pSC101 transduction is size-dependent and is apparently independent of recombination with the donor chromosome. It seems likely that the phage packages a multimer of plasmid DNA, as seen in all others systems examined (Viret *et al.* 1991). We believe that generation of the pWSK30 multimer is mechanistically different than the generation of the concatemer for pBR322. The most likely mechanism for pWSK30 involves interplasmidic recombination to give a large multimeric circle (Figure 1B). According to our model, all plasmids can undergo such recombination events. However, the phage requires 44 kb of DNA to package. Therefore, the larger the plasmid, the fewer recombination events are required to give a sufficiently large circle. The pSC101 derivatives are present in approximately eight copies per cell. Therefore, in the case of pWKS30 (5.4 kb), even if all of the plasmids recombine, then the resulting multimer is only 43 kb and is insufficient for packaging. If the size of the plasmid is increased to 10.4 kb (pJS225), then the large concatemer could potentially be 83 kb and is now packageable. This apparent transition between efficient and nonefficient transduction can be seen in the graph in Figure 2.

The formation of the putative pSC101 circular multimers by interplasmidic recombination is supported by the fact that a mutation in *recF* reduces transduction of pWKS30 derivatives approximately 2.5-fold but does not significantly affect transduction of pBR322. Thus RecF is involved, but not absolutely required, for creation of the packageable pSC101 species, but is not significantly involved in the formation of the pBR322 concatemer. This result is consistent with two different mechanisms being involved in the transduction of pBR322 *vs.* pSC101 derivatives.

Absence of RecD in the donor causes a dramatic increase in the transduction of pSC101 derivatives. Whether this is a result of amplifying the predominant pathway involved in the formation of packageable substrates or inducing a new pathway is unclear. Is the pSC101 derivative now being packaged via rolling circle replication in the *recD* strain? We think this is unlikely because of the fact that transduction via integration into the donor chromosome still represents ~10% of the events. This is in contrast to the analogous pBR322 derivatives that have homology with the chromosome.

In this case, transduction of the integrant represents a very small fraction of the total. Indeed, the fact that both mechanisms of transduction of the pSC101 derivative are equally increased in the *recD* donor suggests that both are products of recombinational events initiated by double strand breaks.

RecA is involved in the formation of all of the packageable substrates. A donor *recA* mutation reduces transduction of both pBR322 and the pSC101 derivatives approximately five- to 10-fold. This does not distinguish formation of the pBR322 concatemer and the pSC101 multimer. However, the *recA* mutation completely blocks plasmid transduction via integration into the donor chromosome. These results support the hypothesis that two distinct mechanisms are involved in the transduction of pSC101 derivatives that share homology with the bacterial chromosome.

We do not mean to imply that pBR322 cannot be transduced by the same mechanisms as pSC101. Indeed, in the case of the pBR322 derivatives, pBAM326 and pBAM362, that contain 5 kb of homology with the chromosome, transduction via integration into the chromosome occurs at the same rate as for the analogous pWKS30 derivative pJS225, when compared to transduction of the chromosomal marker. However, in contrast to pJS225, the pBR322 derivative plasmids can also be transduced by a much more efficient mechanism: generation of a concatemer by rolling circle replication.

When any of the plasmid transducing particles are injected into the recipient, the plasmids must recircularize to be stably maintained. GARZON *et al.* (1995) have shown that the recircularization of a pBR328 concatemer can be mediated by either RecA or Erf, produced from a co-infecting phage particle. They also have shown that RecB is required to resolve both the plasmid concatemer as well as a plasmid integrated into the P22 phage genome by homologous recombination. However, RecB is not required for circularization of P22 phage DNA. They argue that recircularization of the plasmid requires recombination within internal repeats, whereas circularization of the phage must take place on the terminally redundant ends of the phage genome. This implies that recircularization of the pBR328 plasmid concatemer by recombination at the ends of the fragment does not lead to stabile plasmid inheritance, perhaps because a circular multimer is unstable (SUMMERS and SHERRATT 1984).

In the experiments presented here, the pSC101 plasmids are capable of recircularizing in the absence of RecA. Although this is relatively efficient at an MOI of 0.2, the experiments presented here do not directly address the potential role of Erf in this process. The plasmid can recircularize when transduced either as an integrant in a chromosomal fragment or as a putative concatemer. However, our results indicate that in the absence of RecA, the concatemer is approximately five-fold more likely to give rise to stabile plasmid than the transduced integrant. The difference between these molecules is that the integrated plasmid has one region of duplication that is internal to the molecule, whereas the concatemer has a number of tandem duplications both within the molecule as well as on the ends. The fact that the concatemer is more efficient implies that much of the productive recircularization occurs close to the ends, giving rise to a circular multimer that, in apparent contrast to pBR328, leads to stabile inheritance of the plasmid.

Most of the plasmids, including sufficiently large pSC101 derivatives, pBR322, or pBR328, are transduced with an efficiency that is equal to or greater than a chromosomal marker. When the pSC101 derivatives are transduced integrated into the donor chromosome, the transducing fragment that remains after plasmid resolution recombines with the recipient chromosome ~18% of the time. This is similar to previous results showing that ~10% of standard transducing fragments recombine into the recipient chromosome (EBEL-TSIPIS *et al.* 1972). This implies that the majority of plasmids that are injected into the recipient resolve and replicate. It follows that although the overall transduction of the plasmids can exceed that of a chromosomal marker, the frequency is in large part due to the events in the recipient. Thus, a plasmid that is transduced at the same frequency as a chromosomal marker is packaged at a frequency of only 10–20% of the chromosomal marker, suggesting that the chromosome is the far superior packaging substrate.

In the uninfected cell, the plasmid pJS225 recombines with the chromosome at a frequency of 1/6000, as measured by the number of plasmids that obtain the chromosomal Kn^r marker. However, when we select for transduction of the chromosomal Kn^r marker, the plasmid integrant is cotransduced at ~5%. If the plasmid is present at eight copies per cell, then the integrated plasmid represents ~0.6% of the total plasmid population. This implies that this plasmid-chromosome recombination event, and perhaps recombination in general, is increased at least 37-fold in the P22 infected cell. Note that the recombination between the plasmid and the chromosome is RecA dependent and, therefore, this increased recombination cannot be due solely to phage recombination proteins.

Is low-copy plasmid transduction a feasible method of allelic exchange? Given the fact that recombination is dramatically increased in the donor, plasmid transduction is an ideal method to move alleles from the chromosome to the plasmid. Depending on the size of the plasmid, recombinants are a significant fraction of the transduced plasmids. Thus, even if the recombinant plasmid must be identified by restriction mapping, for example, it is feasible. Moving alleles from the plasmid to the chromosome is slightly more problematic. This is because the plasmid is efficiently transduced indepen-

dently of the chromosome and, even when the plasmid is transduced as an integrant, the resulting transducing particle does not always recombine with the recipient chromosome. Moreover, these transduction events are phenotypically indistinguishable. These complications can be avoided by selecting for chromosomal transduction from a plasmid-bearing strain. For example, we have efficiently moved the Mu*dJ* (Kn^r) insertions in plasmids pAL205 and pAL215 into the chromosome by the following method. We constructed a strain that contains a Tn*10d*Tc in the chromosome that is genetically linked to, but not in the chromosomal fragment that corresponds to the insert in the plasmids. Each of the plasmids was introduced into this strain and lysates were grown and used to transduce the wild-type recipient strain for Tc^r, selecting for transduction of the chromosomal marker. Approximately 3% of the transductants also inherited a plasmid that had integrated into the donor chromosome during phage propagation. Of the plasmid-bearing transductants, up to 50% contain the Mu*dJ* recombined into the recipient chromosome. The actual percentage is dependent on the position of the Mu*dJ* in the chromosomal insert. Even if the frequency is 50%, these strains can be dramatically enriched for by pooling the Tc^r, Kn^r transductants, making a P22HT lysate, and transducing the wild-type strain for Tc^r and subsequently screening for Kn^r and Ap^s. Any Mu*dJ* that was in the chromosome in the donor is transduced at high frequency depending on the linkage of the Tn*10d*Tc. Thus, the two steps required to exchange alleles from plasmid to chromosome (a double recombination event with the chromosome and subsequent loss of the plasmid) are facilitated by performing two concurrent P22 transductions.

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