JOURNAL OF BACTERIOLOGY, Nov. 1993, p. 7086–7091
0021-9193/93/217086-06$02.00/0
Copyright © 1993, American Society for Microbiology
Vol. 175, No. 21

Bacteriophage P22 Transduction of Integrated Plasmids: Single-Step Cloning of *Salmonella typhimurium* Gene Fusions

MICHAEL J. MAHAN,†* JAMES M. SLAUCH, AND JOHN J. MEKALANOS

Department of Microbiology and Molecular Genetics, Harvard Medical School, Boston, Massachusetts 02115

Received 14 June 1993/Accepted 4 September 1993

Transcriptional fusions to *Salmonella typhimurium* chromosomal genes were constructed by integration of a suicide fusion vector into the chromosome by homologous recombination with random cloned chromosomal fragments. We describe here a transductional method using the generalized transducing phage of *S. typhimurium*, P22, to clone these fusions directly from the bacterial chromosome, in a single step, without the use of restriction enzymes. In this transduction, the phage packages the chromosomal fragment containing the integrated plasmid. Once introduced into the recipient, the plasmid circularizes by homologous recombination between the duplicated region determined by the cloned fragment. Although RecA mediates the majority of these events, the plasmid can circularize in a *recA* recipient. However, in this case, the event occurs at a much lower frequency and only when the transduction is done at a high multiplicity of infection. In addition to integrated fusion constructs, we also show that autonomously replicating low-copy-number plasmids can be transduced. In this case, transduction is dependent on homologous recombination between the plasmid and the donor chromosome via cloned sequences, in which the transducing particle effectively traps the integrated plasmid.

The use of gene fusions has greatly facilitated the analysis of gene regulation, protein structure, and membrane topology, etc. (19). Recently, we described a method to identify genes that are specifically induced when a pathogenic bacterium is infecting its host (9). The method involves the selection of particular transcriptional fusions to bacterial operons induced during the infection process.

To construct such transcriptional fusions to *Salmonella typhimurium* chromosomal genes, random fragments of *Salmonella* chromosomal DNA were cloned into the broad-host-range suicide fusion vector pIVET1, 5′ to the promoterless *purA-lacZY* genes (Fig. 1A). Some of these cloned fragments contained bacterial promoters in the proper orientation to drive the *purA-lacZY* genes, generating operon fusions. Autonomous replication of pIVET1 derivatives is dependent on the Pi replication protein that must be supplied in *trans*. Therefore, introduction of these fusion constructs into an *S. typhimurium* strain that does not contain plasmid replication functions results in integration of the fusion plasmids into the bacterial chromosome by homologous recombination with the cloned fragments. The product of this integration event generates a chromosomal duplication in which two copies of *S. typhimurium* material flank pIVET1 plasmid sequences at the site of cloned homology (Fig. 1A). The integrated plasmid is organized so that one promoter drives the *purA-lacZY* genes and the other promoter drives a wild-type copy of the gene or operon defined by the cloned fragment. After selection for the fusions of interest, the constructs had to be recovered from the bacterial chromosome for subsequent analysis. Using the generalized transducing phage of *S. typhimurium*, P22, we have devised a single-step method to clone these fusions directly from the bacterial chromosome by transduction of the integrated fusion plasmid into a strain competent for plasmid replication.

* Corresponding author.
† Present address: Department of Biological Sciences, University of California, Santa Barbara, CA 93106.

Construction of *Salmonella* F′ λ*pir*. Our model plasmid system, pIVET1, is a derivative of the broad-host-range suicide vector pGP704, the replication of which is dependent on the Pi replication protein, which can be delivered in *trans* (10). To date, this plasmid has been replicated in various *Escherichia coli* strains, e.g., in strains that harbor a λ lysogen containing a cloned *pir* gene, which encodes the Pi replication protein (7). Thus, our first step was to construct a *S. typhimurium* strain that is capable of supporting autonomous replication of the plasmid.

E. coli SK383 λ*pir* harbors the *pir* gene (encoding the Pi protein) carried on a specialized λi^{434} transducing phage lysogenized at *att*λ in the bacterial chromosome (7). This strain was grown overnight in Luria broth containing 10 mM $MgSO_4$, and the spontaneously released phage present in the supernatant were used to make lysogens in strain ND26 (Δ*gal-bio*), which is deleted for the chromosomal *att*λ and contains F′100-12 (*gal*$^+$ *bio*$^+$ *att*λ$^+$). Briefly, 0.1 ml of supernatant was mixed with 0.1 ml of an overnight culture of ND26. After 10 min at room temperature, the mixture was streaked on a Luria broth plate that had been spread with approximately 10^9 of the clear selector phage, λi^{434}cI. Candidate lysogens were tested for (i) 434 immunity, (ii) the ability to support replication of the Pi-dependent replicon pGP704, and (iii) the ability to transfer F′100-12 λ*pir* by conjugation. F′100-12 λ*pir* was transferred from the ND26 derivative into a *galE496* derivative of the wild-type *S. typhimurium* strain, ATCC 10428, by conjugal transfer, selecting Gal$^+$ Val^r (*E. coli* strains are valine sensitive [22]). This strain, JS30, was tested for the ability to support the autonomous replication of pGP704. (Strains used in this study are described in Table 1.)

Single-step cloning strategy. Phage P22 grown on the fusion strain was used to transduce a recipient strain, JS30, which contains the Pi replication protein required for replication of the plasmid. Presumably, the transducing phage introduces the integrated fusion plasmid as a linear piece of DNA into the recipient cell (Fig. 1B). Once the chromosomal fragment is introduced into the recipient, the plasmid can recircularize by

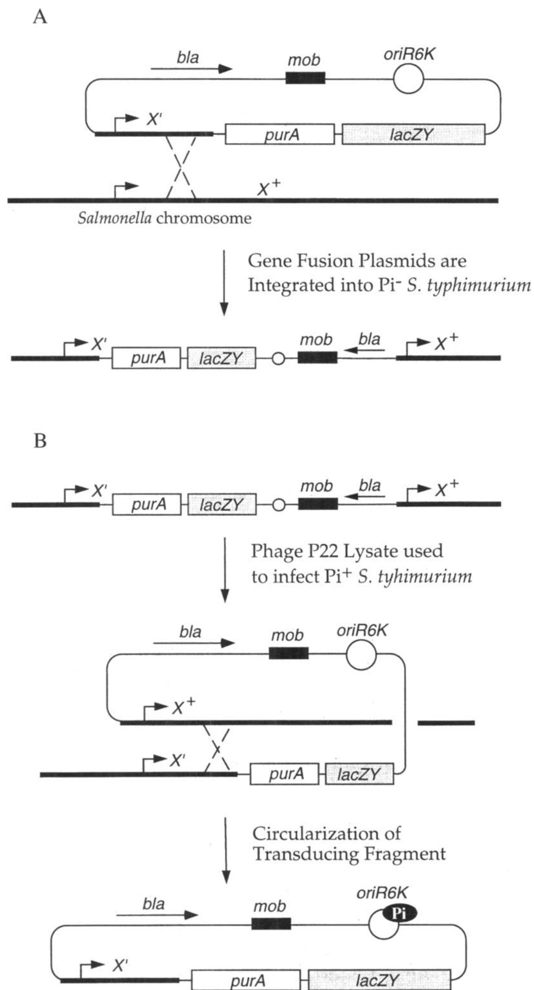

FIG. 1. Single-step cloning of gene fusions by phage P22 transduction. pIVET1 is a suicide vector whose autonomous replication is dependent on the presence of the Pi replication protein, which can be provided in *trans* (9). (A) Construction of single-copy operon fusions by chromosomal integration of the suicide plasmid. pIVET1 contains promoterless *purA* and *lacZY* genes to which random *S. typhimurium* *Sau*3AI restriction DNA fragments were cloned into the *Bgl*II site, 5′ to the *purA-lacZY* genes. Selection for ampicillin resistance in a strain that lacks the Pi replication protein demands integration of the plasmid into the bacterial chromosome. The product of the integration event generates a duplication in which one promoter drives the *purA-lacZY* fusion and the other promoter drives a wild-type copy of the gene or operon defined by the fusion. (B) Transductional cloning event. Phage P22 grown on the integrated gene fusion was used to infect a recipient cell that contains the Pi replication protein. Selection for ampicillin resistance results in the recircularization of the plasmid, using the duplicated *S. typhimurium* sequence as a source of homology, cloning the fusion of interest.

homologous recombination between the duplicated regions defined by the cloned *S. typhimurium* DNA. The circularized fragment then replicates as a plasmid in the presence of the Pi replication protein, resulting in the cloned fusion of interest. Alternatively, the transduced fragment can initially recombine into the bacterial chromosome and subsequently recombine out as a circular fragment. In either case, the only stable event in a Pi^+ background is one in which the plasmid replicates autonomously, presumably because it is deleterious to the cell to have an active plasmid replication origin integrated into the chromosome (1).

Mechanism of transductional cloning. We performed several experiments to address the mechanism of the transductional cloning event. Note that in every case, the efficiency of the plasmid cloning event is internally controlled by normalizing to the ability to transduce $lysA^+$ and repair a *lysA565*::Tn*10* chromosomal marker present in the recipient strain, MT191. This normalization is necessary to account for variations in both the ability of phage P22 to grow on any particular donor strain and the ability of any given strain to act as a recipient.

First, we determined whether the transductional cloning event could occur in a *recA* recipient strain. Table 2 shows that the integrated plasmid gene fusion in strain MT1042 (pMJM11 integrated) can be cloned by transduction into a *recA* Pi^+ recipient strain, MT189. As judged by restriction analysis, these cloned plasmids are identical to those isolated after transductional cloning into an isogenic $recA^+$ recipient, MT190 (data not shown). However, the frequency of cloned fusions obtained in a *recA* strain was severely reduced. Moreover, the cloning event occurred only at a multiplicity of infection greater than 1. The high-multiplicity of infection dependence in a *recA* recipient is a characteristic of a two-fragment transduction event in which, presumably, one transducing fragment contains the integrated fusion and the other fragment encodes recombination functions that result in circularization of the fusion plasmid in the recipient cell.

One candidate for the putative recombination function is the RecA protein produced from a transducing particle containing the $recA^+$ gene from the donor. To test this possibility, we determined whether the frequency of the transductional cloning event was dependent on the donor strain carrying $recA^+$. P22 phage grown on isogenic *recA* and $recA^+$ integrated fusion strains, MT1041 and MT1042, were used as donors to transduce isogenic *recA* and $recA^+$ recipient strains, MT189 and MT191 (*lysA565*::Tn*10*), to ampicillin resistance. Table 2 indicates that transductional cloning of the integrated fusion plasmid into a *recA* strain is not dependent on the donor strain carrying $recA^+$. These results suggest that the proposed two-fragment recombination function is provided by a function other than $RecA^+$ from the donor cell.

We suggest that the proposed recombination function is supplied not by another transducing particle but rather by a phage particle. Upon introduction of phage P22 DNA into a cell, the phage particle is circularized by a homologous recombination event between the terminally redundant ends (13, 20). This event is mediated by a phage-encoded recombination function, Erf (essential recombination function) (2, 4, 14). We propose that Erf may also mediate circularization of the plasmid sequences, explaining the dependence of the event on a high multiplicity of infection.

Transductional cloning of mutagenized bacterial sequences. Once we isolated integrated fusions, we wanted to isolate insertion mutations in the genes defined by the transcriptional fusions. Thus, we have expanded on the above technique to mutagenize the integrated fusion plasmids directly in the bacterial chromosome. Mu*d*-Cm insertion elements are defective Mu phages that contain a chloramphenicol resistance determinant (obtained from T. Elliot). A pool of chromosomal Mu*d*-Cm insertions was generated in the wild-type *S. typhimurium* strain, ATCC 14028, according to methods described previously (6, 9). P22 phage grown on the resultant pool of Mu*d*-Cm insertions was used to transduce recipient strains containing the integrated fusions to chloramphenicol and ampicillin resistance (to avoid replacement of the integrated

TABLE 1. Bacterial strains[a]

Strain	Genotype	Source or reference
S. typhimurium		
ATCC 14028	Wild type	CDC 6516-60
JS30	*galE496 bio-106*::Tn*10*/F′100-12 λ*pir* (*gal*$^+$ *bio*$^+$)	This study
MT12	*lysA565*::Tn*10*	This study
MT189	*recA1 galE496*/F′100-12 λ*pir* (*gal*$^+$ *bio*$^+$)	This study
MT191	*lysA565*::Tn*10 galE496*/F′100-12 λ*pir* (*gal*$^+$ *bio*$^+$)	This study
TT16528	*hisD9953*::Mu*d*-Cm *hisA9941*::MudI	T. Elliot from J. Roth
MT879	*recA1 galE496*/pMJM11 F′100-12 λ*pir* (*gal*$^+$ *bio*$^+$)	This study
MT880	*galE496*/pMJM11 F′100-12 λ*pir* (*gal*$^+$ *bio*$^+$)	This study
MT1038	*recA1 galE496*/pGP704 F′ 100-12 λ*pir* (*gal*$^+$ *bio*$^+$)	This study
MT1039	*galE496*/F′100-12 λ*pir* (*gal*$^+$ *bio*$^+$) pGP704	This study
MT1041	DUP2902[Φ(*iviI′-purA*$^+$*-lacZ*$^+$*Y*$^+$)*1**pIVET1* (*iviI*$^+$)] *recA1 srl-202*::Tn*10d*-Cm	This study
MT1042	DUP2902[Φ(*ivil′-purA*$^+$*-lacZ*$^+$*Y*$^+$)*1**pIVET1* (*ivil*$^+$)] *srl-202*::Tn*10d*-Cm	This study
E. coli		
ND26	Δ(*gal-chl*)*34 his-87 relA1 rpsL181*/F′100-12 λ*pir* (*gal*$^+$ *bio*$^+$)	11
SK838 λ*pir*	F$^-$ *gal lacMS286* φ80d*lacBK1 mtl xyl argE3 his ilu thi* Str^r *dam-4* λ*pir*	7

[a] All bacterial strains derived in this laboratory are derivatives of *S. typhimurium* ATCC 14028 (CDC 6516-60). The nomenclature used for chromosomal rearrangements is as described previously (3, 5, 8, 16).

fusion; Fig. 2A). This results in the mutagenesis, by homologous recombination, of only bacterial and not plasmid sequences in the recipient strain because the initial pool of Mu*d*-Cm insertions was generated in the wild-type strain, ATCC 14028, which does not contain pIVET1 sequences. Phage P22 grown on the pool of Ap^r Cm^r transductants was used to transduce a *recA* Pi$^+$ recipient strain, MT189. Selection for ampicillin resistance results in the recircularization of the plasmid. The Ap^r transductants were subsequently scored for chloramphenicol resistance on rich plates containing the chromogenic substrate 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal). Such Ap^r Cm^r transductants contain the transposon in the cloned bacterial sequences. The advantage of using a *recA* recipient is that subsequent to the initial circularization of the plasmid, there is presumably no recombination with the recipient bacterial chromosome, eliminating the possibility of allelic exchange between the cloned plasmid and the recipient chromosome. To determine whether the Mu*d*-Cm insertion mutations were polar on the operons defined by the cloned gene fusions, the Ap^r Cm^r transductants were scored for decreased expression of the *lacZY* genes present on the vector sequences, turning the colony from blue to light blue. These insertion mutations, by definition, reduce the expression of the operons defined by the fusion.

Once we had isolated an insertion mutation in an operon defined by the fusion, we wanted to recombine this insertion mutation into an otherwise wild-type strain. The resulting strain could then be tested for a phenotype conferred by the insertion. Thus, the mutagenized plasmids were electroporated into the wild-type strain, ATCC 14028, which does not contain the Pi replication protein. Chloramphenicol-resistant transformants were screened for both ampicillin sensitivity and white colony color on rich plates containing X-Gal. Such Cm^r Ap^s white transformants require a double recombination event resulting in a strain containing the Mu*d*-Cm insertion in an otherwise wild-type chromosome (Fig. 2B). The inheritance of the transposon results in a null phenotype if it lands in a structural gene and it is also polar on any genes downstream in the operon. One can now test the phenotype(s) associated with the loss of such transcripts in an otherwise wild-type background.

Transduction of autonomously replicating low-copy-number plasmids. We also tested whether gene fusions present on autonomously replicating plasmids could be transduced in a fashion similar to that when the fusions were integrated in the chromosome. Note that for reasons that are unclear, we have observed a 10-fold reduction in the ability of phage P22 to grow on donor strains that contain autonomously replicating pGP704 plasmid derivatives; this reduction is exacerbated if the donor strain is a *recA* mutant (>10^2-fold). Phage P22 was grown on MT880 (*recA*$^+$ Pi$^+$), which contains an autonomously replicating plasmid gene fusion (pMJM11). Table 3 shows that the transduction of pMJM11 from a *recA*$^+$ donor strain (MT880) into a *recA*$^+$ strain (MT191) occurs at a frequency similar to that of a *lysA*$^+$ chromosomal marker. In addition, the transduction of pMJM11 from a *recA*$^+$ donor

TABLE 2. RecA dependence of cloning integrated gene fusions by phage P22 transduction

Recipient strain; selected marker	No. of transductants obtained with donor strain[a]:							
	MT1042 (integrated pMJM11; *recA*$^+$)				MT1041 (integrated pMJM11; *recA*)			
	10^0	10^{-1}	10^{-2}	10^{-3}	10^0	10^{-1}	10^{-2}	10^{-3}
MT189 (*recA1*); Ap^r	1,312	4	0	0	1,912	13	0	0
MT191 (*recA*$^+$); Ap^r	>5,000	1,760	143	21	>5,000	1,832	232	22
MT191 (*lysA565*::Tn*10 recA*$^+$); LysA$^+$	>5,000	2,061	197	18	>5,000	2,874	327	28

[a] The high-frequency generalized transducing bacteriophage P22 mutant HT 105/1 *int-201* (18) was used for all transductional crosses. P22 phage stock used in these experiments was grown on MT12 (*lysA565*::Tn*10*) to prevent carryover of *lysA*$^+$ to the recipient strain. The 10^0 phage P22 lysates grown on the donor strains were 4.8×10^9 PFU/ml for MT1042 and 1.8×10^{10} PFU/ml for MT1041; 10^{-1}, 10^{-2}, and 10^{-3} represent serial dilutions of undiluted phage lysate. For each transduction, 0.1 ml of an overnight culture grown in complex medium (ca. 2×10^9 to 4×10^9 CFU/ml) was used as a recipient of 0.1 ml of transducing phage and plated directly on selective plates.

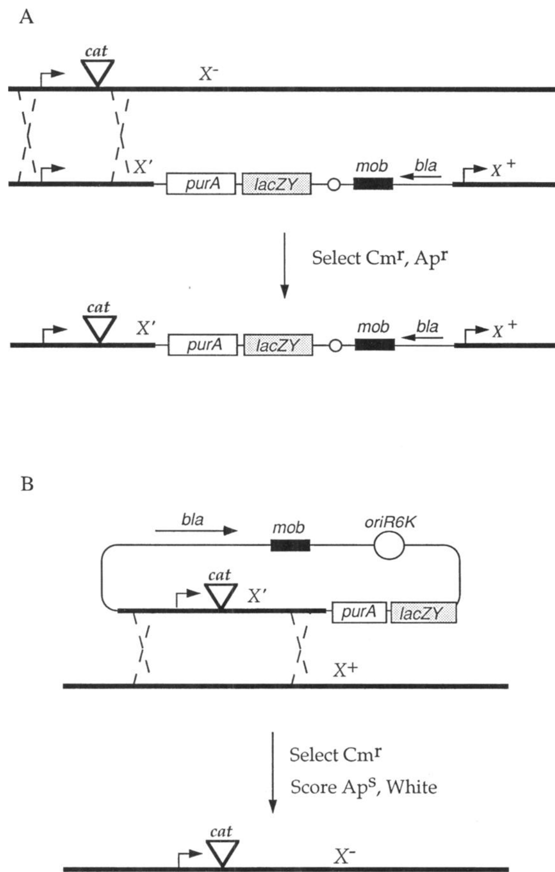

FIG. 2. Insertional mutagenesis. (A) A pool of Mu*d*-Cm transposon insertions was generated in the *S. typhimurium* wild-type strain, ATCC 14028. Phage P22 grown on this pool of Mu*d*-Cm insertions was used to transduce an integrated fusion strain to chloramphenicol and ampicillin resistance (to maintain the plasmid fusion). The resulting Cm^r Ap^r transductants have Mu*d*-Cm insertions in only bacterial and not plasmid sequences. The mutagenized gene fusions were then cloned by transduction into strain MT189 (*recA* Pi^+), selecting ampicillin resistance (see text). Cloned gene fusions, which contained a Mu*d*-Cm insertion element that decreased the expression of the *lacZY* genes, were scored as Cm^r recombinants that were light blue on rich medium containing X-Gal (see text). These polar insertion mutations, by definition, contain Mu*d*-Cm insertion elements in the operons defined by the fusions. (B) The polar Mu*d*-Cm insertion-bearing plasmids were electroporated into the wild-type *S. typhimurium* strain, ATCC 14028. A double recombination event resulting in the inheritance of the insertion in an otherwise wild-type chromosome was scored as Cm^r Ap^s transductants that formed white colonies on rich medium containing X-Gal.

strain, MT880, into a *recA* recipient strain, MT189, is severely reduced compared with that into a *recA*$^+$ recipient strain, MT191. These results are similar to those obtained when the plasmid was integrated into the chromosome (Table 2).

We then tested whether transduction of the autonomously replicating plasmid was dependent on RecA in the donor strain. Table 3 indicates that transduction of pMJM11 from a *recA* donor strain (MT879) into a *recA*$^+$ recipient was reduced at least 16-fold compared with the transduction of a *lysA*$^+$ chromosomal marker; this sharp reduction was not seen when the donor was a *recA*$^+$ strain (MT880). Note that the observed 16-fold reduction is a minimum estimate of the RecA dependence of plasmid transduction because of the severe inability to grow phage P22 on *recA* strains that contain an autonomously replicating pGP704 plasmid derivative. This RecA dependence is in contrast to when the plasmid was integrated into the chromosome (Table 2), suggesting that a homologous recombination event is required in the donor in order to transduce the autonomously replicating plasmid.

The homologous recombination event required in the donor to allow transduction of autonomously replicating plasmids could be either recombination between individual plasmids or recombination between the plasmid and the donor chromosome. To distinguish between these possibilities we attempted to transduce the parent plasmid, pGP704, which contains no chromosomal sequences. Table 3 shows that the transduction of pGP704 from a *recA*$^+$ donor strain, MT1039, into a recipient strain, MT191 (*lysA*::Tn*10* *recA*$^+$), is reduced at least 800-fold compared with transduction of a *lysA*$^+$ chromosomal marker. In contrast, transduction of pMJM11, which contains chromosomal homology, from a *recA*$^+$ donor, MT880, shows only a modest (three- to fivefold) reduction compared with that of the *lysA*$^+$ chromosomal marker. Taken together, the results in Table 3 indicate that transduction of pGP704-derived plasmids is highly dependent on homologous recombination between the plasmid and the chromosome in the donor strain, suggesting a chromosomal intermediate during the plasmid transduction process.

Implications and applications. Plasmid transduction by phage P22 has been previously reported. In the case of P22 that is wild type for packaging, it was shown that pBR322-based plasmids could be transduced if the plasmid contained either homology to phage P22 (12) or a cloned *pac* site, the sequence at which P22 packaging is initiated (17). In the first case, transduction was dependent on homologous recombination in the donor strain and the phage apparently packages a cointegrate of the plasmid and the phage genome (12). In the second case, transduction was not dependent on a recombinationally proficient donor. Presumably, P22 phage packages, beginning at a *pac* site, a concatamer of the *pac*-containing plasmid generated by rolling-circle replication (17). In contrast, the high-transducing mutant of P22, HT (18), which contains a mutation that effectively reduces the stringency of the *pac* site, can transduce pBR322 independent of any sequence homology (15). It has been suggested that the phage is packaging a concatamer of plasmid generated by rolling-circle replication as in the case of *pac*-containing plasmids. Indeed, the transduction of these high-copy-number plasmids is significantly higher than transduction of chromosomal markers from the same lysate. Phage-mediated induction of rolling-circle plasmid replication and packaging of the resulting concatemer has been observed in a variety of systems (reviewed in reference 23).

All of the above plasmid transduction events occur independently of the bacterial chromosome in the donor. In contrast, Trun and Silhavy (21) reported the transduction of low-copy-number plasmids containing cloned chromosomal fragments in *E. coli* by the generalized transducing phage P1. In this case, transduction is apparently dependent on recombination of the plasmid with the chromosome. The phage packages the chromosomal fragment, essentially trapping the plasmid integrate. This is apparently analogous to the events described here. In our experiments, transduction of the low-copy-number plasmids requires recombination with the chromosome in the donor.

Why do low-copy-number plasmids require integration into the chromosome in order to be transduced, whereas pBR322 derivatives do not? We hypothesize that it may simply be a matter of having a sufficient amount of DNA to allow packag-

TABLE 3. Homology dependence of cloning autonomously replicating plasmid gene fusions by phage P22 transduction

Recipient strain; selected marker	No. of transductants obtained with donor strain[a]: MT880 (pMJM11 *recA*$^+$)			MT879[b] (pMJM11 *recA1*)			MT1039 (pGP704 *recA*$^+$)			MT1038 (pGP704 *recA1*)		
	10^0	10^{-1}	10^{-2}	10^0	10^{-1}	10^{-2}	10^0	10^{-1}	10^{-2}	10^0	10^{-1}	10^{-2}
MT189 (*recA1*); Ap^r	0	0	0	0	0	0	2	0	0	0	0	0
MT191 (*recA*$^+$); Ap^r	207	27	1	0	0	0	2	1	0	0	0	0
MT191 (*lysA565*::Tn*10* *recA*$^+$); LysA$^+$	356	78	5	16	0	0	~3,000	808	86	1,202	156	13

[a] The high-frequency generalized transducing bacteriophage P22 mutant HT 105/1 *int-201* (18) was used for all transductional crosses. P22 phage stock used in these experiments was grown on MT12 (*lysA565*::Tn*10*) to prevent carryover of *lysA*$^+$ to the recipient strain. The 10^0 phage P22 lysates grown on the donor strains were 4.2 $\times 10^7$ PFU/ml for MT880, 1.7 $\times 10^5$ PFU/ml for MT879, 1.6 $\times 10^9$ PFU/ml for MT1039, and 1.7 $\times 10^8$ PFU/ml for MT1038; 10^{-1} and 10^{-2} represent serial dilutions of undiluted phage lysate. For each transduction, 0.1 ml of an overnight culture grown in complex medium (ca. 2×10^9 to 4×10^9 CFU/ml) was used as a recipient of 0.1 ml of transducing phage and plated directly on selective plates.

[b] When MT879 was used as an undiluted (10^0) phage P22 lysate, 0.5 ml of this donor phage was mixed with 0.1 ml of recipient.

ing by P22HT. If P22 induces rolling-circle replication of pBR322-based plasmids, this would provide a sufficient length of DNA to allow packaging independent of plasmid monomer size. On the other hand, perhaps the phage is not capable of inducing rolling-circle replication of the low-copy-number plasmids, and therefore, a suitable substrate for packaging is not available. However, if the plasmid recombines into the chromosome, then this would provide a sufficient length of DNA to allow packaging. The phage packages the chromosomal fragment containing the integrated plasmid.

The technique described here should not be limited to suicide plasmids but should also be useful for transduction of any low-copy-number, autonomously replicating plasmids, analogous to the events described by Trun and Silhavy (21). Because the transduction of low-copy-number plasmids apparently requires integration of the plasmid into the chromosome by homologous recombination, this provides a means to facilitate allelic exchange between plasmid and chromosomal sequences. For example, to clone a chromosomal marker, one can introduce a low-copy-number plasmid carrying a DNA fragment that contains the wild-type sequence into the mutant strain and subsequently transduce the plasmid. Because the plasmid has to integrate into the chromosome in order to be transduced, this effectively traps a recombinational intermediate between the chromosome and the plasmid required for allelic exchange. Thus, a large fraction of the transduced plasmids should contain the original chromosomal mutation, depending on the position of the mutation with respect to the cloned sequences. Note that this cross is also affected by the allele present in the recipient chromosome in that the DNA fragment containing the integrated plasmid can initially recombine with the recipient chromosome prior to plasmid circularization, thus affecting the ultimate frequency of obtaining the desired clone. In certain circumstances, this triallelic cross (two alleles from the donor and one in the recipient) may be desirable. Indeed, many variations of this scheme are possible, making this a generally applicable technique.

This work was supported by National Institutes of Health grant AI26289 (to J.J.M.), National Research Science award AI08245 (to M.J.M.), and Damon Runyon-Walter Winchell Cancer Research Fund grant DRG-1016 (to J.M.S).

REFERENCES

1. **Bernander, R., A. Merryweather, and K. Nordstrom.** 1989. Overinitiation of replication of the *Escherichia coli* chromosome from an integrated runaway-replication derivative of plasmid R1. J. Bacteriol. **171:**674–683.
2. **Botstein, D., and M. J. Matz.** 1970. A recombination function essential to the growth of bacteriophage P22. J. Mol. Biol. **54:**417–440.
3. **Chumley, F. G., and J. R. Roth.** 1980. Rearrangement of the bacterial chromosome using Tn*10* as a region of homology. Genetics **94:**1–14.
4. **Fenton, A. C., and A. R. Poteete.** 1984. Genetic analysis of the *erf* region of the bacteriophage P22 chromosome. Virology **134:**148–160.
5. **Hughes, K. T., and J. R. Roth.** 1985. Directed formation of deletions and duplications using Mu*d* (Ap, *lac*). Genetics **109:**263–282.
6. **Hughes, K. T., and J. R. Roth.** 1988. Transitory cis complementation: a method for providing transposition functions to defective transposons. Genetics **119:**9–12.
7. **Kolter, R., M. Inuzuka, and D. R. Helinski.** 1978. Trans-complementation-dependent replication of a low molecular weight origin fragment from plasmid R6K. Cell **15:**1199–1208.
8. **Mahan, M. J., and J. R. Roth.** 1991. Ability of bacterial chromosome segment to invert is dictated by included material rather than flanking sequence. Genetics **129:**1021–1032.
9. **Mahan, M. J., J. M. Slauch, and J. J. Mekalanos.** 1993. Selection of bacterial virulence genes that are specifically induced in host tissues. Science **259:**696–688.
10. **Miller, V. L., and J. J. Mekalanos.** 1988. A novel suicide vector and its use in construction of insertion mutations: osmoregulation of outer membrane proteins and virulence determinants in *Vibrio cholerae* requires *toxR*. J. Bacteriol. **170:**2575–2583.
11. **Ohtsubo, E., and M.-T. Hsu.** 1978. Electron microscope heteroduplex studies of sequence relations among plasmids of *Escherichia coli*: structure of F100, F152, and F8 and mapping of the *Escherichia coli* chromosomal region *fep-supE-gal-att*λ*-uvrB* J. Bacteriol. **134:**778–794.
12. **Orbach, M. J., and E. N. Jackson.** 1982. Transfer of chimeric plasmids among *Salmonella typhimurium* strains by P22 transduction. J. Bacteriol. **149:**985–994.
13. **Poteete, A. R.** 1988. Bacteriophage P22, p. 647–682. *In* R. Calender (ed.), The bacteriophages, vol. 2. Plenum Press, New York.
14. **Poteete, A. R., and A. C. Fenton.** 1983. DNA-binding properties of the Erf protein of bacteriophage P22. J. Mol. Biol. **163:**257–275.
15. **Sanderson, K. E., and J. R. Roth.** 1983. Linkage map of *Salmonella typhimurium*, edition VI. Microbiol. Rev. **47:**410–453.
16. **Schmid, M. B., and J. R. Roth.** 1980. Circularization of transducing fragments: a mechanism for adding segments to the bacterial chromosome. Genetics **94:**15–29.
17. **Schmidt, C., and H. Schmieger.** 1984. Selective transduction of recombinant plasmids with cloned *pac* sites by *Salmonella* phage P22. Mol. Gen. Genet. **196:**123–128.
18. **Schmieger, H.** 1972. Phage P22-mutants with increased or decreased transduction abilities. Mol. Gen. Genet. **119:**75–88.
19. **Slauch, J. M., and T. J. Silhavy.** 1991. Genetic fusions as experimental tools. Methods Enzymol. **204:**213–248.
20. **Susskind, M. M., and D. Botstein.** 1978. Molecular genetics of

bacteriophage P22. Microbiol. Rev. **42**:385–413.
21. **Trun, N. J., and T. J. Silhavy.** 1987. Characterization and in vivo cloning of *prlC*, a suppressor of signal sequence mutations in *Escherichia coli* K12. Genetics **116**:513–521.
22. **Umbarger, H. E.** 1987. Biosynthesis of branched-chain amino acids, p. 352–367. *In* F. C. Neidhart, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology. American Society for Microbiology, Washington, D.C.
23. **Viret, J.-F., A. Bravo, and J. C. Alonso.** 1991. Recombination-dependent concatemeric plasmid replication. Microbiol. Rev. **55**: 675–683.