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New prime plasmids from *Pseudomonas aeruginosa*

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SUMMARY

pMO1111, a derivative of FP110 carrying a Tn*1* insert, has been used to generate two plasmid primes carrying *Pseudomonas aeruginosa* chromosome. pMO1112 has been shown to carry the *pro-82*$^+$ gene and pMO1113 carries *argH*$^+$ and *lys-12*$^+$. pMO1112 is considerably more stable than pMO1113 in a *recA*$^+$ background.

1. INTRODUCTION

Plasmids carrying sections of bacterial chromosomes are a valuable tool in microbial genetics. The isolation of F primes (Low, 1972; Cannon, Dixon & Postgate, 1976) or R primes (Hedges, Jacob & Crawford, 1977; Holloway, 1978; Holloway *et al.* 1980; Johnston, Setchell & Beringer, 1978; Kiss *et al.* 1980) indicates that *in vivo* cloning could be a natural function of plasmids but to date only two types of plasmids have been shown to have this property – F, and certain IncP-1 plasmids, notably R68·45. Up to 30 % of strains of *Pseudomonas aeruginosa* isolated from hospitals carry FP plasmids which can mobilize bacterial chromosome but do not carry any resistance determinants (Dean, Royle & Morgan, 1979). Here we describe the isolation of two plasmid primes from pMO1111, a derivative of FP110 to which Tn*1* has been transposed. FP110 is a plasmid with chromosome mobilizing ability (Cma) identified in a hospital strain of *P. aeruginosa* (Royle & Holloway, 1980).

From incompatibility, host range, phage inhibition and entry exclusion data, FP110 has been shown to be related to R18-1, RP1-1 and R56Be (Holloway & Richmond, 1973; Michel-Briand, Stanisich & Jouvenot, 1977; Royle & Holloway, 1980). The source of Tn*1* was R18, a plasmid closely related or identical to RP4 (Chandler & Krishnapillai, 1974*b*; Holloway & Richmond, 1973). R18 and RP4 have been shown to be identical in restriction enzyme fragment pattern (H. Stokes, R. Moore & V. Krishnapillai, unpublished data).

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2. MATERIALS AND METHODS

The media and experimental procedures used were the same as those described in previous papers from this laboratory (Holloway, 1978; Pemberton & Holloway, 1973; Royle & Holloway, 1980). The markers of strains used in these experiments are shown in Fig. 1.

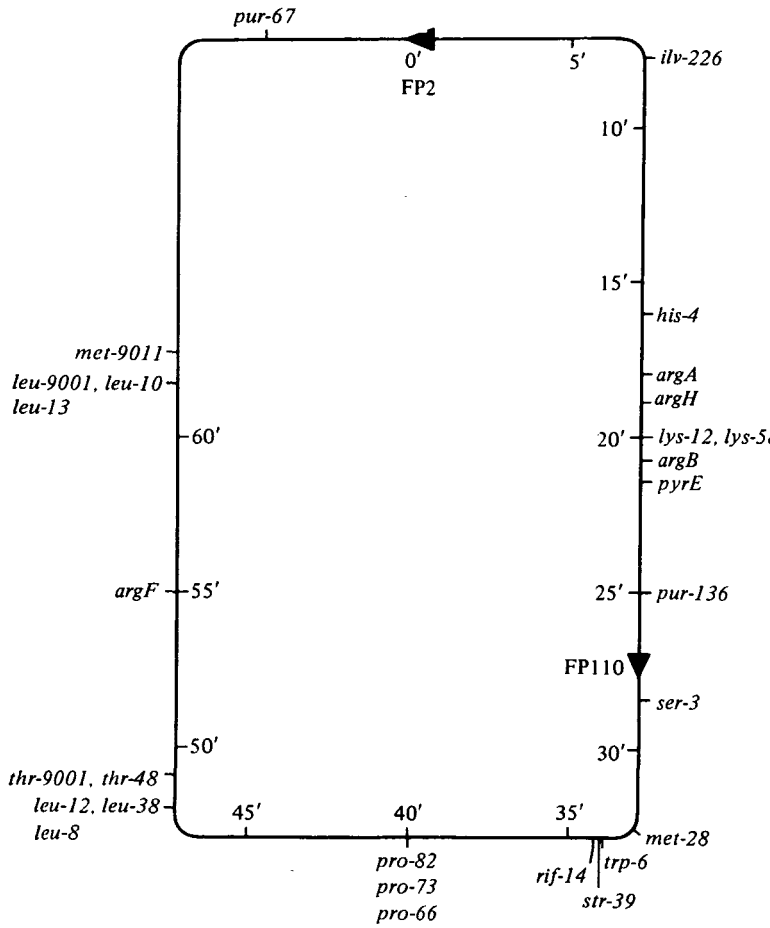

Fig. 1. Chromosome map of *P. aeruginosa* PAO showing the markers used in this paper. This map is derived from those previously published (Holloway *et al.* 1979; Holloway & Royle, 1980). Abbreviations as in Table 1.

3. RESULTS

(i) *Isolation of plasmid primes*

The technique/used for isolating primes from pMO1111 was essentially that described for *Escherichia coli* (Low, 1972; Ou & Anderson, 1976) and *P. aeruginosa* (Holloway, 1978). The donor strain carrying the Cma plasmid is mated to a *recA*

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Table 1. *Genotypes of strains used in this study*

The abbreviations used here and in the text are:
(*arg*, arginine requirement; *bla*, β-lactamase production; Cma, chromosome mobilizing ability; *his*, histidine requirement; *ilv*, requirement for isoleucine and valine; *leu*, leucine requirement; *lys*, lysine requirement; *met*, methionine requirement; *pro*, proline requirement; *pur*, adenine requirement; *rec*, recombination deficiency; *rif*, rifampicin resistance; *ser*, serine requirement; *str*, streptomycin resistance; *thr*, threonine requirement; *trp*, tryptophan requirement.)

Strain	Genotype	Reference
PAO1	Prototrophic	Holloway, 1969
PAO25 (pMO1111)	*argF*, *leu-10*, *bla* (formerly PAO25 FP110::Tn1)	Royle & Holloway, 1980
PAO140	*pro-66*, *thr-9001*, *argF32*, *leu-9001*, *met-9011*, *pur-67*	Holloway strain collection
PAO220	*met-28*, *trp-6*, *lys-12*, *his-4*, *pro-82*	Holloway strain collection
PAO222	*met-28*, *trp-6*, *lys-12*, *his-4*, *pro-82*, *ilv-226*	Haas & Holloway, 1976
PAO224	*met-28*, *trp-6*, *lys-12*, *his-4*, *pro-82*, *leu-12*	Holloway strain collection
PAO227	*met-28*, *trp-6*, *lys-12*, *his-4*, *pro-82*, *ilv-226*, *leu-13*	Watson & Holloway, 1978
PAO260	*met-28*, *trp-6*, *lys-12*, *his-4*, *pro-82*, *ilv-226*, *rif-14*	Holloway, 1978
PAO372	*argH32*, *lys-58*	Haas *et al.* 1977
PAO878	*pur-136*, *leu-8*, *pro-73*	Holloway strain collection
PAO2003	*argH32*, *str-39*, *rec-2*	Chandler & Krishnapillai, 1974*a*
PAO2828	*ser-3*, *pro-73*	Holloway strain collection
PTO66	*trp-6*, *lys-12*, *his-4*, *pro-82*, *ilv-1118*, *rec-102*	V. Krishnapillai, personal comm.
PAT2228 (FP2)	*leu-2104*, *lys-1115*, *trp-3114*, *pur-1118*, *pro-2108*, *ilv-1118*, *his-2117*, *thr-1109*, *rec-102*	J. Watson, personal comm.

recipient and selection made for a chromosomal marker. Any progeny that arise presumably do so by the acquisition of plasmids carrying a fragment of donor bacterial chromosome which has not integrated into the chromosome of the recipient.

PTO66 was used as a recipient in these crosses. This strain, constructed by V. Krishnapillai, is a recombinant from the cross PAT2228 (FP2-2) × PAO220. It requires histidine, lysine, isoleucine, valine, tryptophan and proline and its recombination-deficient phenotype is similar to the RecA-like mutation *rec-2* of PAO2003 (Chandler & Krishnapillai, 1974*a*) so that PTO66 is essentially a PAO strain carrying a *rec* mutation and six auxotrophic markers. The only other available PAO *rec* strain, PAO2003, only carries *argH*.

With PAO25 (pMO1111) (Royle & Holloway, 1980) as the donor and, using direct plate mating (Holloway, 1978), selection was made in turn for each of the auxotrophic markers carried by PTO66. Recombinants occurred at a frequency of less than 10^{-10} per donor cell. From the recombinants obtained, two different plasmid primes were identified; pMO1112, which was isolated when selection was made for *pro-82*$^+$ and pMO1113 when selection was for *lys-12*$^+$ (see Fig. 1). The strains PTO66 (pMO1112) and PTO66 (pMO1113) were found to have retained the plasmid properties of phage B39 resistance, carbenicillin resistance and Tra$^+$ ability for CB^r (Royle & Holloway, 1980). Evidence that these plasmids carry both plasmic and bacterial chromosomal DNA was obtained as follows.

(ii) *Cotransfer of plasmid and bacterial chromosome markers*

The ability of each plasmid prime to transfer both chromosomal and plasmid markers together was shown in matings either by selecting the bacterial marker (*pro-82*$^+$ for pMO1112 and lys-*12*$^+$ for pMO1113) and measuring the coinheritance of the plasmid determined carbenicillin resistance or alternatively by selecting for carbenicillin resistance and scoring the coinheritance of the unselected bacterial marker. The results are shown in Table 2, and provide strong evidence that the plasmid and bacterial markers are transferred together as part of the same DNA fragment. It was possible to obtain some idea of the size of the chromosomal fragment of pMO1113 by matings to strains carrying markers known to be situated close to *lys-12* (Haas *et al.* 1977). As shown in Table 3, pMO1113 can transfer both *argH* and *lys-12*. However *argA* and *argB* are not transferred, in contrast to results with R′PA1, a prime derivative of R68·45 which carries all of these markers (Holloway, 1978). This suggests that pMO1113 carries a length of bacterial chromosome 0·5–2·0 min long terminating between *lys-12* and *argB* at one end, and between *argH* and *argA* at the other end.

Because there are no suitable markers situated close to *pro-82* on the PAO chromosome, it was not possible to demonstrate the transfer of other bacterial markers by this plasmid prime.

(iii) *Demonstration of partial diploidy in bacteria carrying the prime plasmid*

The partial diploidy of the chromosomal region carried by pMO1113 in PAO strains carrying this plasmid was shown for the *argH* locus. pMO1113 was transferred to PAO2003 (*argH32*, *rec-2*) the latter marker maintaining the stability of pMO1113 by preventing the integration of the chromosomal fragment carried by the plasmid. It has been shown that *lys-12* and *argH* are 45 % cotransducible by

Table 2. *Marker transfer properties of pMO1112 and pMO1113 to PAO260*

Donor strain	Phenotype selected	Transfer frequency per donor cell	% coinheritance of unselected phenotype
PTO66 (pMO1112)	Pro$^+$	$5{\cdot}0 \times 10^{-2}$	91 (CB^r)
	Cb^r	$5{\cdot}0 \times 10^{-2}$	78 (Pro$^+$)
PTO66 (pMO1113)	Lys$^+$	$1{\cdot}2 \times 10^{-2}$	70 (CB^r)
	Cb^r	$1{\cdot}2 \times 10^{-2}$	62 (Lys$^+$)

Mating was by mixing equal volumes of exponential phase donor culture with the stationary phase recipient PAO260 and immediately plating 0·2 ml aliquots on appropriate selective media. One hundred recombinants for each selected marker were scored for coinheritance of the unselected marker as indicated in the table.

Table 3. *Transfer frequency by pMO1113 of various chromosomal markers*

Selected marker	Map location (minutes from FP2 origin)	Transfer frequency per donor cell
his-4$^+$	16	$< 10^{-7}$
argA$^+$	18	$< 10^{-7}$
argH$^+$	19	$1{\cdot}0 \times 10^{-3}$
lys-12$^+$	20	$1{\cdot}0 \times 10^{-3}$
argB$^+$	20	$< 10^{-7}$
pyrE$^+$	21	$< 10^{-7}$

PTO66 (pMO1113) was mated to strains containing the markers indicated. The standard plate mating technique was used (Stanisich & Holloway, 1969).

phage F116L (Haas *et al.* 1977). PAO2003 (pMO1113) was found to be phenotypically prototrophic, indicating the likely presence of the *argH*$^+$ allele. The presence of the *argH* allele in this strain was shown by growing the transducing phage F116L on PAO2003 (pMO1113) and transducing into PAO222 as recipient with selection for *lys-12*$^+$. Cotransduction of *lys-12*$^+$ and *argH* was found to be 66 %. The presence of the *argH*$^+$ allele on the plasmid in PAO2003 (pMO1113) was further shown using the same transducing phage preparation and PAO372 as the recipient. Selection was made on minimal medium plus lysine for *argH*$^+$ transductants, these being found at a frequency of 10^{-6}/p.f.u. which is the same as that found when PAO1 is the donor. Thus PAO2003 (pMO1113) is a partial diploid for at least the *argH* locus. Again, because there are no other markers cotransducible with *pro-82*, it was not possible to demonstrate partial diploidy of the *pro-82* region in strains carrying pMO1112.

(iv) *Stability of pMO1112 and pMO1113*

Plasmid primes are usually unstable in a RecA$^+$ host (Dixon, Cannon & Kondorosi, 1976; Low, 1972; Mergeay & Gerits, 1978; Holloway, 1978). pMO1113 is unstable as shown when PTO66 (pMO1113) is patch-mated to PAO260 on nutrient agar for 18 h. Transconjugant colonies selected on nutrient agar plus carbenicillin and rifampicin were purified and, after being grown in 2 ml nutrient broth plus 0·4 % KNO_3 in Wasserman tubes, were each tested for their retention

Table 4. *Ability of pMO1112 to transfer* pro-82$^+$ *using* rec$^+$ *and* rec *donors and recipients. For each cross* pro-82$^+$ *is the marker selected*

Donor	Recipient	Transfer frequency *pro-82*$^+$/donor cell	% coinheritance CB^r
PAO2003 (pMO1112)	PTO66	$2{\cdot}0 \times 10^{-2}$	100
PAO2828 (pMO1112)	PTO66	$5{\cdot}0 \times 10^{-2}$	100
PAO2828 (pMO1112)	PAO260	$6{\cdot}1 \times 10^{-2}$	100
PAO878 (pMO1112)	PTO66	$2{\cdot}2 \times 10^{-2}$	100
PAO878 (pMO1112)	PAO260	$5{\cdot}6 \times 10^{-2}$	100

Plate matings were carried out by the standard technique.

of pMO1113 by the ability to transfer *arg*$^+$ to PAO372. Of 50 transconjugants tested, only one had the ability to transfer *arg*$^+$ at high frequency showing that pMO1113 is highly unstable in a Rec$^+$ background. One of the forty-nine clones which did not transfer *arg*$^+$ at high frequency was tested for Cma activity by plate mating it to PAO260. Recombinant frequencies of up to 5×10^{-5}/donor cell for *ilv-226*$^+$, *his-4*$^+$ and *lys-12*$^+$ were observed, these frequencies being the same as those found for these markers with FP110 and pMO1111. It is reasonable to assume that the pMO1111 component of pMO1113 is retained intact on loss of the bacterial fragment of the plasmid prime.

By contrast, pMO1112 is much more stable than pMO1113. PTO66 (pMO1112) was patch mated for 18 hours on nutrient agar to PAO260 and transconjugant colonies selected on nutrient agar plus carbenicillin and rifampicin as described above with pMO1113. Broth cultures of the transconjugants were patch mated overnight to PAO140 on minimal medium plus threonine, arginine, leucine, methionine and purine, to detect the transfer of the *pro*$^+$ marker. Of 96 colonies tested, 88 had retained the ability to transfer *pro*$^+$ at high frequency, indicating a much higher degree of stability for pMO1112 in a Rec$^+$ host than was found for pMO1113.

To further examine the stability of pMO1112 the strain PAO2828 (pMO1112) was constructed and mated to PAO227. The transfer frequency of *pro-82*$^+$ was $6{\cdot}0 \times 10^{-2}$/donor cell, the same as that for the Cb^r marker, but every other chromosomal marker was transferred at a frequency less than 10^{-4}/donor cell, suggesting that pMO1112 was being maintained intact in the *rec*$^+$ host PAO2828. By means of matings with PAO2828 (pMO1112) (Rec$^+$) and PAO2003 (pMO1112) (Rec$^-$) as donors and PTO66 (Rec$^-$) and PAO260 (Rec$^+$) as recipients, it was shown that

pMO1112 was capable of transferring the *pro-82*$^+$ marker with equal efficiency to and from both Rec$^+$ and Rec$^-$ hosts of *P. aeruginosa* (Table 4). The high frequency of co-transfer of both the plasmid and chromosomal markers in these matings provides additional evidence for the prime nature of pMO1112.

The stability of pMO1112 in RecA$^+$ hosts permits an indirect demonstration that bacterial chromosome material carried by pMO1112 can become integrated into the chromosome if separated from the rest of the plasmid. PAO2828 (pMO1112) was used to propagate the transducing phage F116L and with this phage, using PAO224(FP2) as recipient, selection was made for *pro*$^+$ transductants. The transduction and selection of transductants was carried out at 43 °C which prevents vegetative multiplication of F116L and hence transductants do not become lysogenic for this phage. F116L, which can transduce plasmids of up to approximately 44 megadaltons (Stanisich & Bennett, 1976) should not transduce pMO1111 and indeed F116L propagated on PAO25 (pMO1111) did not transduce carbenicillin resistance to PAO1.

A randomly selected *pro*$^+$ transductant was plate mated with PAO140, and selection made for *pro*$^+$ recombinants. These were scored for coinheritance of *thr*$^+$ (unselected). In the same manner, recombinants were selected for *thr*$^+$ and scored for coinheritance of the unselected *pro*$^+$ marker. Selection for *pro*$^+$, gave 31 % *pro*$^+$ *thr*$^+$, and selection for thr$^+$ gave 66 % *thr*$^+$ *pro*$^+$. These coinheritance values are close to those expected for these chromosomal markers in FP2 crosses (data not shown). These results show that the *pro*$^+$ allele carried by pMO1112 is capable of integrating into the PAO chromosome. It is not known what aspect of the structure of pMO1112 prevents integration of the chromosomal fragment carried by pMO1112 into the host chromosome. With pMO1113, the chromosomal fragment carrying *argH*$^+$ and *lys-12*$^+$ is readily integrated in a *recA*$^+$ host, as shown above.

(v) *Chromosome mobilizing properties of pMO1112 and pMO1113*

It is known that F-primes can promote polarized chromosome transfer from a preferred origin (Low, 1972; Pittard & Adelberg, 1963). R primes constructed from RP4 by *in vitro* techniques also show polarized chromosome transfer from a preferred origin in both *E. coli* and *R. meliloti* (Barth, 1979; Julliot & Boistard, 1979).

For both pMO1112 and pMO1113, matings with PAO recipients to determine their chromosome mobilizing ability were carried out. With each plasmid, the pattern of marker mobilization was the same as with FP110 and pMO1111, namely a major origin in the 28 min region of the PAO chromosome. Apart from the markers transferred at high frequency for each plasmid as described above, each plasmid had no other preferred site of origin for chromosome transfer (data not shown). These two prime plasmids differ in this respect from the plasmids referred to above. Similar properties to pMO1112 and pMO1113 have also been found with R'PA1 and R primes derived from R68·45 in *P. aeruginosa* (Holloway, 1978; Royle & Holloway, unpublished data).

Another feature of F primes in *E. coli* is their ability to generate Hfr donors

(Low, 1972). Using similar techniques to those found to be successful in *E. coli*, 'Hfr' forms of pMO1112 and pMO1113 were sought. In such strains, the plasmids or part thereof, would be integrated into the chromosome, giving high frequencies of transfer for certain chromosomal markers and loss of transfer of the plasmid alone. Experiments to find such donor strains included the transfer of pMO1113 to PAO372, with joint selection of Cb^r and *argH*$^+$ and tests of the transconjugants for Cma. Such a selection could lead to the identification of transconjugants in which all or part of the plasmid had integrated into the chromosome to create donor strains with high frequency chromosome transfer from a preferred origin. None were found with either pMO1112 or pMO1113, but in the latter case the instability of this plasmid in RecA$^+$ hosts may have rendered the possibility of detecting such integration more unlikely.

4. DISCUSSION

These two plasmid primes differ from F primes in *E. coli* in their inability to mobilize chromosome from any preferred site other than that of the parent plasmids FP110 and pMO1111 and in their failure to generate Hfr donor derivatives. Different plasmid primes, derived from different plasmids and various bacterial genera show a range of properties with respect to their stability. For example, R'PA1, which is derived from R68·45 and carries a fragment of *P. aeruginosa* chromosome, is highly unstable in *P. aeruginosa* RecA$^+$ hosts, although highly stable in *P. putida* strains (Holloway, 1978; A. Morgan, unpublished data). pGY1, also a derivative of R68·45 but carrying a segment of *Rh. meliloti* chromosome, is stable in RecA$^+$ *Rh. meliloti* (Kiss *et al.* 1980). pMO1112 and pMO1113 apparently only differ with respect to the region of the bacterial chromosome that they carry, but there is a marked difference in the degree of stability in a RecA$^+$ host. The data on the transduction of the *pro-82*$^+$ allele from pMO1112 show that there is no overall barrier to reintegration of the bacterial chromosome fragment carried by that plasmid prime. An alternative view is that stability or, perhaps more correctly, retention of the bacterial chromosome fragment in the plasmid prime, could be a function of the parent plasmid DNA. Attempts to compare the physical nature of the DNA of FP110, pMO1111, pMO1112 and pMO1113 have been frustrated by our inability to isolate the DNA of any of the plasmids, or indeed to isolate the DNA of any FP plasmid so far examined.

It is possible that the stability of pMO1112 has increased (probably by selection for a stable variant) with its passage through various hosts and this is seen by comparing the coinheritance data of *pro-82*$^+$ and CB^r in Tables 2 and 4. A stable plasmid prime such as pMO1112 is similar in some respects to FP39 (Pemberton & Holloway, 1973). This naturally occurring plasmid carries information which suppresses (or complements) *P. aeruginosa* PAO strains mutant at the *leu-38* locus to produce a Leu$^+$ phenotype. It has not been possible to demonstrate that FP39 actually carries bacterial chromosomal DNA and the inability to isolate FP39 plasmid DNA prevents determination of whether the Leu$^+$ phenotype is due to

bacterial chromosomal DNA or whether a suppressor gene on the plasmid genome is responsible. Primes of FP39 have not yet been sought. However despite extensive efforts, attempts to find plasmid primes with the more widely used plasmid FP2 were unsuccessful.

Attempts to find other examples of plasmid primes from pMO1111 by selection for other bacterial markers have also been unsuccessful. The regions of the PAO chromosome for which plasmid primes derived from pMO1111 have been demonstrated, have been dictated by the markers available in the *rec* strain PTO666. There does not appear to be any relationship between the *pro-82* and *lys-12* regions and the site of origin of chromosome transfer (28′) demonstrated for FP110 and pMO1111. Our data do not show if the ability to form plasmid primes is a property of the FP110 genome alone or whether the presence of Tn*1* has created the conditions necessary for their isolation. As selective plasmid markers were not available with FP110, the carbenicillin resistance conferred by Tn*1* was a necessary part of the evidence needed to confirm that plasmid primes were being produced. In view of the recent work of Finger & Krishnapillai (1980) in which the transposons Tn*1*, Tn*7* and Tn*501* were used to mark 20 FP plasmids with resistance markers, it should now be possible to examine the role of such transposons in generating plasmid primes.

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