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Linkage Map of *Pseudomonas aeruginosa* PAT

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The locations of new markers relative to markers previously mapped on the chromosome of *Pseudomonas aeruginosa* strain PAT were defined by generalized transduction with phages F116L and F1083. Although the marker orders of the various marker groups were deduced mainly from the results of two-factor crosses, the locations of a number of markers were confirmed by three-factor crosses. A linkage map of the chromosome of *P. aeruginosa* PAT was constructed which shows the relative locations of 50 genes. From the available data, the linkage maps of *P. aeruginosa* strains PAO and PAT appear to be similar.

Most of the genetic knowledge of *Pseudomonas aeruginosa* has come from the study of two strains, PAO (originally strain 1) and PAT2 (previously strain 2). Both are prototrophs and aeruginocinogenic, and they have been earlier described in detail (9, 10, 34). Chromosome mapping studies in *P. aeruginosa* have largely been confined to strain PAO (2, 3, 6-8, 20-22, 26, 27). Genetic analysis of strain PAO was first made possible by the introduction of the conjugative plasmid FP2 from the donor strain (14, 27) and the development of an interrupted mating technique (21, 22, 27). Chromosome mapping in *P. aeruginosa* has been greatly facilitated by the isolation of the R-plasmid variant R68.45, which has enhanced chromosome-mobilizing ability (6, 7).

Genetic analysis of particular regions of the strain PAO chromosome has been achieved by generalized transduction, using phage F116 (11), G101 (15), or F116L (8, 19, 26). Although genetic circularity of the PAO chromosome has not yet been demonstrated, Pemberton (25) has shown that the chromosome is physically circular, with an estimated molecular weight of 2.1×10^9.

Initial attempts to eliminate the FP2 plasmid from strain PAT were unsuccessful (12, 28). Consequently, the first genetic studies in this strain used F116-mediated transduction. These studies showed that loci controlling the same biosynthetic pathway are generally not closely linked in *P. aeruginosa* and also revealed close linkage of a number of different loci (4, 13, 31, 32).

Stanisich and Holloway (30) isolated a line of strain PAT which acted as a recipient in crosses with PAT donors and which could itself be converted to the donor phenotype by the acquisition of FP2. This recipient line of PAT was used by Stanisich and Holloway (29) to show that the IncP-1 plasmid R68 and the IncP-10 plasmid R91 were able to mobilize chromosomal markers at frequencies of 10^{-4} to 10^{-6} per donor cell. Recent studies have shown that these R plasmids mobilize the PAT chromosome from distinct origins. The polarity of chromosome mobilization by R68 and R91 is opposite to that by FP2, and mapping studies with these plasmids and R68.45 have provided linkage evidence for chromosomal circularity in strain PAT (34).

In this paper we present the results of further mapping studies using generalized transduction. These studies have resulted in the definition of a chromosome map of *P. aeruginosa* strain PAT showing the locations of 50 genes.

(A preliminary account of these results was presented at the Third International Symposium on Antibiotic Resistance, Smolenice, Czechoslovakia, June 1976.)

MATERIALS AND METHODS

Bacterial and bacteriophage strains. The bacterial strains used in this study are shown in Table 1. All PAT recipient (FP^-) strains were derived from the prototroph PAT964 (30); all donor ($FP2^+$) strains were derived from PAT2. Phages F116L (19) and F1083 (P. Chandler, Ph.D. thesis, Monash University, Clayton, Victoria, Australia, 1975) were used for transductions.

Media. Nutrient broth, heart infusion agar (HIA), layer agar, and minimal medium (MM) have been described previously (33, 34). The antibiotics carbenicillin (as Pyopen, Beecham), streptomycin (Sigma), spectinomycin (Upjohn), rifampin (Ciba-Geigy), fusidic acid (sodium salt, Smith Kline & French), nalidixic acid (Sterling), and mitomycin (Kyowa) were added to HIA or MM at the concentrations indicated. Amino acids, purines, and pyrimidines were added to MM as required to a final concentration of 1 mM, except isoleucine, which was used at 0.5 mM. Stocks of amino acid solutions (50 mM) were kept over chloroform. *p*-Fluorophenylalanine was added to MM to give a final concentration of 1 mg/ml.

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TABLE 1. *Bacterial strains used in this study*

Strain	Genotype[a]	Derivation[b]	Reference
PAT2	Prototroph, FP2$^+$	Clinical isolate	9
PAT404	*his-2404 strA100*, FP^{2+}	PAT2, NG for *his-2404*, spontaneous for *strA100*	30
PAT919	*his-2404 arg-1104 strA100*	PAT900, NG	30
PAT900	*his-2404 strA100*	ICR 191[c] treatment of PAT404 to remove FP2 plasmid	30
PAT964	Prototroph	Recombinant from PAT2 × PAT919	30
PAT967	*met-2105*	PAT964	30
PAT985	*ilv-1106 met-2105*	PAT967	V. A. Stanisich (unpublished data)
PAT1068	*phe-5102* FP2$^+$	PAT2	31
PAT1304	*his-2404 strA100 aerR101*, FP2$^+$	PAT404, NG	P. Chandler (unpublished data)
PAT2001	*leu-2104*	PAT964	34
PAT2066	*leu-2104 met-3121*	PAT2001	This paper
PAT2069	*leu-1108*	PAT964	This paper
PAT2070	*leu-1108 pur-1114 nal-111*	PAT2069	This paper
PAT2090	*leu-2104 met-3121 trp-4112*	PAT2066	This paper
PAT2092	*leu-2104 met-3121 pur-2117*	PAT2066	This paper
PAT2096	*leu-1108 pur-1114 thr-1103 nal-111*	PAT2070	This paper
PAT2097	*leu-1108 pur-1114 thr-1103 thr-2105 nal-111*	PAT2096	This paper
PAT2103	*leu-2104 met-3121 lys-1115 trp-3114*	From PAT2066, intermediate parent strain lost	This paper
PAT2104	*leu-2104 lys-1115 trp-3114*	Spontaneous *met*$^+$ revertant of PAT2103	This paper
PAT2105	*leu-2104 lys-1115 trp-3114 pur-1118*	PAT2104	This paper
PAT2109	*arg-2119*	PAT964	34
PAT2111	*arg-3121*	PAT964	34
PAT2113	*arg-4123*	PAT964	This paper
PAT2117	*arg-6127*	PAT964	This paper
PAT2119	*met-2105 ilv-1106 ser-1105*	PAT985	This paper
PAT2120	*met-2105 ilv-1106 ser-1105 pro-3106*	PAT2119	This paper
PAT2123	*his-2404 strA100 aerR101 arg-3129*	PAT1304	This paper
PAT2124	*his-2404 strA100 aerR101 arg-3129 trp-3115*	PAT2123	This paper
PAT2127	*leu-2104 lys-1115 trp-3114 pur-1118 arg-1130*	PAT2105	33
PAT2128	*leu-2104 lys-1115 trp-3114 pur-1118 arg-2131*	PAT2105	This paper
PAT2131	*leu-2104 lys-1115 trp-3114 pur-1118 arg-5134*	PAT2105	This paper
PAT2134	*leu-2104 lys-1115 trp-3114 pur-1118 arg-2131 nalA114*	PAT2128	This paper
PAT2135	Prototroph, *rifA101*, FP2$^+$	PAT2	This paper
PAT2136	Prototroph, *strA113*, FP2$^+$	PAT2	This paper
PAT2137	Prototroph, *spcA100*, FP2$^+$	PAT2	This paper
PAT2142	*leu-2104 lys-1115 trp-3114 pur-1118 pro-1107*	PAT2105	This paper
PAT2143	*leu-2104 lys-1115 trp-3114 pur-1118 pro-2108*	PAT2105	33
PAT2153	*leu-2104 lys-1115 trp-3114 pur-1118 thr-2106*	PAT2105	33
PAT2154	*leu-2104 lys-1115 trp-3114 pur-1118 thr-3107*	PAT2105	33
PAT2155	*leu-2104 lys-1115 trp-3114 pur-1118 thr-1108*	PAT2105	33
PAT2163	Prototroph, *fusA100*, FP2$^+$	PAT2	This paper
PAT2164	*leu-2104 lys-1115 trp-3114 pro-2108 ilv-1108 pur-1118*	PAT2143	This paper
PAT2166	*leu-2104 lys-1115 trp-3114 pro-2108 ilv-1118 his-1116*	PAT2164	This paper
PAT2167	*leu-2104 lys-1115 trp-3114 pro-2108 ilv-1118 pur-1118 his-2117*	PAT2164	This paper

TABLE 1—*Continued*

Strain	Genotype[a]	Derivation[b]	Reference
PAT2168	*leu-2104 lys-1115 trp-3114 pro-2108 ilv-1118 pur-1118 his-3118*	PAT2164	This paper
PAT2170	*leu-2104 lys-1115 trp-3114 pro-2108 ilv-1118 pur-1118 his-5120*	PAT 2164	This paper
PAT2173	*leu-2104 lys-1115 pro-2108 ilv-1118 pur-1118 his-3118*	*trp*$^+$ transductant of PAT2168[d]	This paper
PAT2177	*leu-2104 lys-1115 trp-3114 pro-2108 ilv-1118 pur-1118 his-2117 thr-1109*	PAT2167	33
PAT2180	*leu-2104, lys-1115 trp-3114 pro-2108 pur-1118 his-5120*	*ilv*$^+$ transductant of PAT2170[d]	This paper
PAT2189	*arg-3121 trp-3117 strA116*	PAT2230	This paper
PAT2190	*leu-2104 met-3121 arg-5140*	PAT2066	This paper
PAT2193	*leu-2104 lys-1115 trp-3114 pur-1118 cys-3105*	PAT2105	This paper
PAT2200	*leu-2104 met-3121 ilv-3122*	Localized mutagenesis of PAT2190, using hydroxylamine-treated F116L on PAT2	This paper
PAT2229	*arg-3121 trp-5116*	PAT2111	This paper
PAT2230	*arg-3121 trp-3117*	PAT2111	This paper
PAT2235	*trp-3117*	*arg*$^+$ transductant of PAT2230[d]	This paper
PAT2246	*leu-2104 lys-1115 trp-3114 thr-2106*	*pur*$^+$ transductant of PAT2153[d]	This paper
PAT2247	*leu-2104 lys-1115 trp-3114 thr-3107*	*pur*$^+$ transductant of PAT2154[d]	This paper
PAT2248	*leu-2104 lys-1115 trp-3114 thr-1108*	*pur*$^+$ transductant of PAT2155[d]	This paper
PAT2249	*arg-4123 his-1123*	PAT2213	This paper
PAT2250	*leu-2104 lys-1115 trp-3114 pur-1118 pro-1107* (Ts)*2104*	PAT2142	This paper
PAT2251	*leu-2104 lys-1115 trp-3114 pur-1118 cys-3105 cbsA100*	PAT2193	J. Fyfe (unpublished data)
PAT2256	*leu-2104 trp-3114 pur-1118 pro-1107* (Ts)*2104*	*lys*$^+$ transductant of PAT2250[d]	This paper
PAT2260	*met-2105 ilv-1106 ser-1105 pro-3106, pyr-1108*	PAT2120	This paper
PAT2261	*leu-2104 lys-1115 trp-3114 pur-1118 pro-2108 ilv-1118 his-2117 thr-1109 ksgA100*	PAT2177	This paper
PAT2262	*leu-2104 lys-1115 pur-1118 pro-2108 ilv-1118 his-2117 thr-1109 ksgA100*	*trp*$^+$ recombinant from mating PAT2261 × PAT404	This paper
2-2004	Prototroph, *fpaA101*, FP2$^+$	PAT2	32

[a] Abbreviations: *aer*, aeruginocin production; *arg*, arginine requirement; *cbs*, carbenicillin hypersensitivity; *cys*, cysteine requirement; *fpa*, *p*-fluorophenylalanine resistance; *fus*, fusidic acid hypersensitivity; *his*, histidine requirement; *ilv*, isoleucine (*ilv-3*) or isoleucine and valine requirement (*ilv-1* and *ilv-2*); *leu*, leucine requirement; *lys*, lysine requirement; *met*, methionine requirement; *nal*, nalidixic acid resistance; *phe*, phenylalanine requirement; *pro*, proline requirement; *pur*, adenine requirement; *pyr*, uracil requirement; *rif*, rifampin resistance; *ser*, serine requirement; *spc*, spectinomycin resistance; *str*, streptomycin resistance; *sup*, suppressor activity; *thr*, threonine (*thr-1*), homoserine (*thr-2*), or threonine and homoserine (*thr-3*) requirement; *trp*, tryptophan requirement; Ts, temperature sensitive for growth at 43°C. Mutations are designated according to the nomenclature of Watson and Holloway (33). The first number after each gene symbol indicates the arbitrary locus designation, whereas the following three numbers refer to the allele number; e.g., *ilv-1106* indicates allele *106* of the *ilv-1* locus. Where a particular allele has not been assigned to a known locus, the allele number only is shown, e.g., *nal-111* (PAT2070). To standardize the nomenclature for strain PAT genes, a number of previously published locus numbers have been changed as follows (each new locus designation is followed by the previous locus designation in parentheses): *met-2 (met-2a), met-3 (met-2b), pur-1 (ade-1), pur-2 (ade-2), thr-1 (thr), thr-2 (hom), trp-3 (trp-3bi), trp-4 (trp-3bii)* (4), and *phe-5* (*pheV*) (32). All strains are FP$^-$ unless stated to be FP2$^+$.

[b] Unless otherwise stated, all mutants were obtained after treatment of the parent strain indicated with ethyl methane sulfonate. NG indicates mutagenesis with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine.

[c] ICR 191, 6-Chloro-9[[3-[(2-chloroethyl)amino]-propyl]amino]-2-methoxyacridine.

[d] In these transductions, the phage was F116L propagated on PAT2.

Isolation of mutants. Auxotrophic mutants were isolated after ethyl methane sulfonate mutagenesis and carbenicillin enrichment as previously described (34). Spontaneous mutants resistant to antibiotics were selected on HIA containing streptomycin (1 mg/ml), spectinomycin (2 mg/ml), rifampin (200 μg/ml), or nalidixic acid (1 mg/ml for *nalA* mutants or 400 μg/ml for *nalB* mutants). Strain PAT2163, which is hypersensitive to fusidic acid, was isolated after ethyl methane sulfonate mutagenesis of PAT2 by replica plating surviving clones to HIA containing 2 mg of fusidic acid per ml.

Transduction. The procedures for preparation of transducing lysates and for transduction have been previously described (19). Between 200 and 500 transductants were scored for each cross, so that failure to detect cotransduction between any two markers was solely within the limits imposed by these numbers. The cotransduction values were averaged in most cases from reciprocal crosses and usually from several different crosses.

Characterization of transductants. Transductants were partially purified by spotting to the same selective medium. After overnight incubation at 37°C, the master plates were replica plates to supplemented MM or HIA containing antibiotics to score for the segregation of unselected auxotrophic or antibiotic-resistant/hypersensitive markers, respectively. Temperature sensitivity was scored by replica plating to HIA or appropriately supplemented MM followed by overnight incubation at 43°C.

Aeruginocinogenicity was scored by replica plating partially purified clones to HIA containing 0.5 μg of mitomycin per ml. After overnight incubation at 37°C, the bulk of the cells were removed by imprinting the plate onto sterile velvet, and the residual cells were killed by inverting the plate over chloroform for 30 min. The plate was then overlaid with 2 ml of layer agar plus 1 ml of nutrient broth and 0.1 ml of an overnight culture of strain WT1040. Aeruginocinogenicity was indicated by clearing of the WT1040 lawn. Strain WT1040 is a wild-type isolate of *P. aeruginosa* which is sensitive to the R-type aeruginocin produced by strain PAT (P. Chandler, unpublished data).

RESULTS

Conjugational mapping studies have revealed the locations of a number of genes on the strain PAT chromosome (34). The loci *his-1* (9 min), *lys-1* (19 min), *ilv-1* (29 min), *trp-3* (36 min), *pur-1* (47 min), and *leu-2* (52 min) have each been found to be closely linked to a number of other markers. The linkage relationships within each of these groups of closely linked markers were examined by transduction. The transductional groups described below were designated according to the map location of one of the above reference loci. Transductional linkage maps were drawn approximately to scale, using the mapping formula of Wu (35), although, strictly, that formula was designed for three-factor crosses and two-factor-cross data was used in the map construction. For these calculations it was assumed that the molecular weight of F116L DNA is the same as that of F116 DNA, previously shown to be 3.8×10^7 (10, 19), and that this amount of DNA is the maximum length which can be transduced. The tabular numerical data and the details of actual crosses are given in the Appendix. The data are given in summary form in Fig. 1 through 6.

Transductional linkage in the 9-min region. In strain PAO, the markers *his-5075* and *cys-5605* are highly cotransducible by F116 (24). In strain PAT, at least five unlinked loci controlling histidine biosynthesis have been identified by transductional analysis (4; J. Watson, Ph.D. thesis, Monash University, Clayton, Victoria, Australia, 1977). The *his-1116* marker was found to be contransducible with *cys-3105*. Transductional analysis has shown that the *cys-3105* allele is not closely linked to the *cys-1* or *cys-2* locus of Fargie and Holloway (4), and hence it represents a new *cys* locus in strain PAT (Watson, Ph.D. thesis). No cotransduction (<1%) was observed between any other *his* and *cys* loci.

A number of mutants of PAT which are hypersensitive to carbenicillin have been isolated (J. Fyfe, unpublished data). One of the alleles conferring this phenotype (*cbsA100*) is also cotransducible with an allele of the *his-1* locus. The relative order of the three loci in this group is suggested by the frequencies of the various classes of transductants derived from the cross donor PAT2251 × recipient PAT2249. Of 200 selected *his-1123*$^+$ transductants, 12 were found to have the donor genotype, namely *his-1123*$^+$ *cys-3105 cbsA100*, whereas none had the genotype *his-1123*$^+$ *cys-3105*$^+$ *cbsA100*. This observation is consistent with the marker order shown in Fig. 1.

Transduction linkage in the 19-min region. Four loci were found to be closely linked to the *lys-1* locus, situated at 19 min on the chromosome (Fig. 2). The *arg-1130* and *arg-5134* alleles were both cotransducible with *lys-1115*. These *arg* alleles are homologous with the *argH* and *argB* mutations, respectively, of strain PAO (8) on the bases of transduction data and

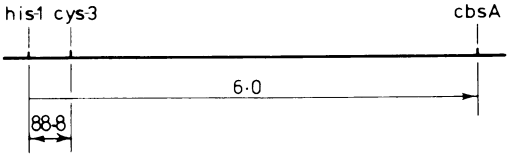

FIG. 1. *Transductional linkage in the 9-min region. Arrowheads indicate unselected markers. Numbers indicate the percentages of cotransduction by F116L.*

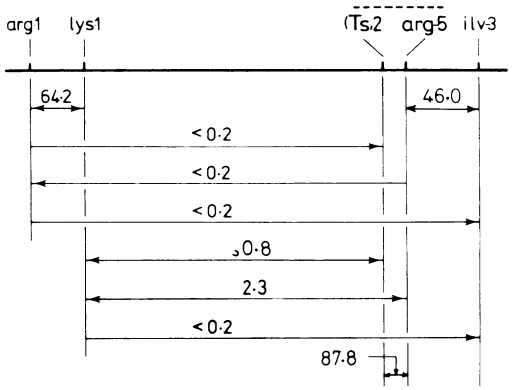

FIG. 2. *Transductional linkage in the 19-min region. Arrowheads indicate unselected markers. The orientation of the markers covered by the dashed line is equivocal. Numbers indicate the percentages of cotransduction by F116L.*

the growth responses to ornithine and citrulline which they confer (Watson, Ph.D. thesis). The result of a cross between the two PAT *arg* mutants indicated that the *arg* alleles are not cotransducible and therefore suggests that the marker order is *arg-1130–lys-1115–arg-5134.*

The *ilv-3122* mutation of PAT2200 confers a requirement for isoleucine alone, suggesting that this strain is threonine dehydratase deficient (5). The *ilv-3122* marker was found to be cotransducible with *arg-5134* but not with *lys-1115* or *arg-1130.* These data therefore suggest the marker order *arg-1130–lys-1115–arg-5134–ilv-3122.*

The temperature-sensitive marker (Ts)*2104* was initially found to be cotransducible with *lys-1115,* but further transductions revealed that (Ts)*2104* was highly cotransducible with *arg-5134* but was not cotransducible with *arg*-1130. Examination of the transductants from the cross donor PAT2256 × recipient PAT2131 revealed that 2 out of 200 selected *lys-1115*$^+$ transductants had the donor genotype, *lys-1115*$^+$ (Ts)*2104 arg-5134*$^+$, whereas none had the recombinant genotype *lys-1115*$^+$ (Ts)*2104*$^+$ *arg-5134*$^+$. Similarly, 3 out of 200 selected *arg-5134*$^+$ transductants were of the donor genotype, whereas none had the above recombinant genotype. These data are consistent with the location of (Ts)*2104* between *lys-1115* and *arg-5134*; however, this has not been confirmed by a three-factor cross involving the (Ts)*2104*, *arg-5134*, and *ilv-3122* alleles.

Transduction linkage in the 29-min region. Transductional mapping studies in strain PAO (26) indicated that the markers *ser-204*, *ilv-202*, *met-28*, *pro-70*, and *pyrB21* were very closely linked in the 28-min region of the chromosome. Strains PAT2120 and PAT2260 were used for transductional mapping of the homologous region of the PAT chromosome. These two strains were derived from PAT985, in which the *ilv-1106* and *met-2105* alleles were found to be very closely linked in FP2-mediated conjugation (V. A. Stanisich, personal communication).

Transductional analyses of PAT2120 showed that *ilv-1106* was cotransducible with the markers *ser-1105*, *met-2105*, and *pro-3106.* The *ser-1105* allele was 0.5% cotransducible with the closely linked *met-2105* and *pro-3106* markers when the latter two markers were individually selected, suggesting that the order of these alleles is *ser-1105–ilv-1106–(met-2105 pro-3106).* The relative order of *met-2105* and *pro-3106* could not be deduced from these data.

The *nalB114* mutation, which determines low-level resistance to nalidixic acid (34) was cotransducible with the *pro-3106* and *met-2105* markers but not with *ilv-1106* or *ser-1105.* This suggests the tentative marker order *ser-1105–ilv-1106–(met-2105 pro-3106)–nalB114.* The relative order of *met-2105* and *pro-3106* with respect to *ilv-1106* and *nalB114* was deduced from an analysis of the transductants derived from the cross donor PAT2134 × recipient PAT2260. Among 500 selected *ilv-1106*$^+$ transductants, 9 were of the genotype *ilv-1106*$^+$ *met-2105*$^+$ *pro-3106*$^+$, whereas 1 transductant had the genotype *ilv-1106*$^+$ *met-2105*$^+$ *pro-3106.* No transductants having the genotype *ilv-1106*$^+$ *met-2105 pro-3106*$^+$ were observed. These observations were consistent with the marker order *ilv-1106–met-2105–pro-3106.* This marker order was also indicated by the fact that 214 out of 500 selected *met-2105*$^+$ transductants from this cross had the genotype *met-2105*$^+$ *pro-3106*$^+$ *nalB114*, whereas none was of the genotype *met-2105*$^+$ *pro-3106 nalB114.* Taken together, these data indicated that the most likely marker order is *ilv-1106–met-2105–pro-3106–nalB114.*

The *pyr-1108* marker of PAT2260 has not been correlated with the *ura* loci defined by Fargie and Holloway (4). This marker was cotransducible with the *pro-3106* and *met-2105* markers. Of 200 selected *met-2105*$^+$ transductants, derived from the cross donor PAT2 × recipient PAT2260, 168 had the donor genotype *met-2105*$^+$ *pro-3106*$^+$ *pyr-1108*$^+$, whereas none had the recombinant genotype *met-2105*$^+$ *pro-3106 pyr-1108*$^+$. Similarly, among 200 selected *pyr-1108*$^+$ transductants, 103 had the donor genotype, whereas none was of the above recombinant genotype. These observations were consistent with the marker order *met-2105–pro-3106–pyr-1108.* The transductional linkages be-

tween *pyr-1108* and the *ilv-1106* and *nalB114* markers have not been determined.

Transduction studies have indicated that the *his-5120* marker of PAT2180 is not closely linked to any of the *his* loci defined by Fargie and Holloway (4) and that this represents a new *his* locus in strain PAT (Watson, Ph.D. thesis). This marker was found to be cotransducible with *ser-1105* and *ilv-1106* but not with *met-2105* or *pro-3106*. Examination of the transductants derived from this cross revealed that 15 out of 500 selected *ser-1105*$^+$ transductants had the donor genotype *ser-1105*$^+$ *his-5120 ilv-1106*$^+$, whereas none had the recombinant genotype *ser-1105*$^+$ *his-5120*$^+$ *ilv-1106*$^+$. Among 500 selected *ilv-1106*$^+$ transductants, 1 was found to have the donor genotype, whereas none had the above recombinant genotype. These observations suggested that the most likely gene order is *ser-1105–his-5120–ilv-1106*.

The transductional linkages between the markers in this region of the chromosome are summarized in Fig. 3. The gene order *pro-3–pyr-1–nalB* is based only on the results of two-factor crosses and has not been confirmed by an appropriate three-factor cross.

Transductional linkage in the 36-min region. The gene order *trp-3–arg-3–fpaA–str* was originally established in strain PAT by Waltho and Holloway (32) from the results of two-factor crosses with phage F116. The linkages between the above loci have been reevaluated in terms of F116L-mediated transduction, and a number of new markers have been found to be located in this linkage group (Fig. 4).

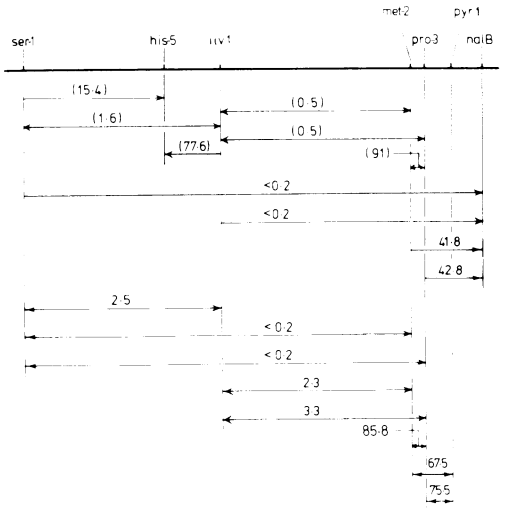

FIG. 3. *Transductional linkage in the 29-min region. Arrowheads indicate unselected markers. Numbers indicate the percentages of cotransduction by F116L or F1083 (in parentheses).*

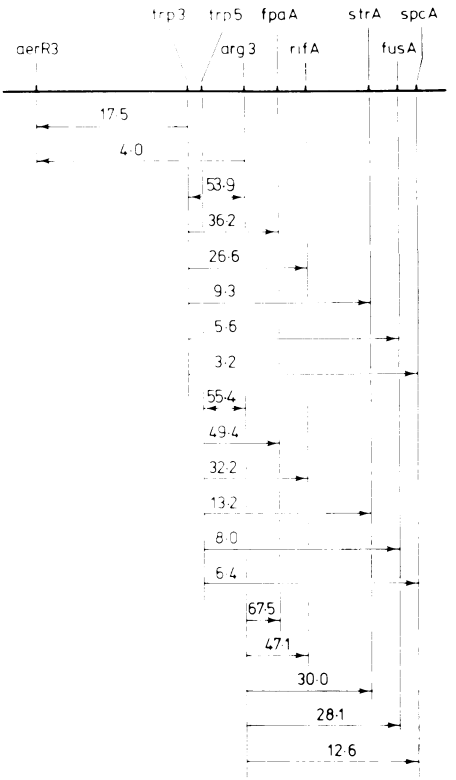

FIG. 4. *Transductional linkage group in the 36-min region. Arrowheads indicate unselected markers. Numbers indicate percentages of cotransduction by F116L.*

Transductional analysis of *trp* mutants of strain PAT indicated that the *trp-3117* and *trp-5116* mutations, which confer different growth responses, are very closely linked (Watson, Ph.D. thesis). The *trp-5116* mutation confers a requirement for anthranilate, indole, or tryptophan, indicating that it is situated in the locus determining anthranilate synthase (1). The average cotransduction frequencies between *arg-3121* and *trp-3117* or *trp-5116* suggested that the relative order of these markers is *trp-3117–trp-5116–arg-3121*, although this has not been confirmed by appropriate three-factor crosses. It should be pointed out that cotransduction values in reciprocal crosses were not always similar. For example, with *trp-5* and *arg-3*, selection for *trp-5*$^+$ gave 74.2, 74.0, 58.8, 55.0, and 62.0% cotransduction with *arg-3* in various crosses, whereas selection for *arg-3*$^+$ gave 46.4, 59.0, 46.6, 36.0, and 42.0% cotransduction with *trp-5*. We have chosen to average these results (55.4%) in the absence of any good explanation for this nonreciprocity. Examples of such nonreciprocal values were found in other regions.

The markers *rifA101*, *fusA100*, and *spcA100*, in addition to *fpaA101* and *strA113* (32), were cotransducible with the above auxotrophic markers. The provisional marker order, based only on these two-factor crosses, is *trp-3117-trp-5116-arg-3121-fpaA101-rifA101-strA113-fusA100-spcA100*. Among 500 selected *trp-3117*$^+$ transductants from the cross donor PAT2262 × recipient PAT2189, 37 had the donor genotype, *trp-3117*$^+$ *arg-3121*$^+$ *strA116*$^+$, whereas only 1 had the recombinant genotype *trp-3117*$^+$ *arg-3121 strA116*$^+$. This observation is consistent with the marker order *trp-3117-arg-3121-strA116*.

The R-type aeruginocin determinant of strain PAT has been shown to map in this region of the chromosome (18). The transductional linkages between the *aer* and the *trp-3* and *arg-3* loci were determined with strain PAT1304 and its derivatives PAT2123 and PAT2124. PAT1304 has been shown to be defective in the production of R-type aeruginocin (16; Chandler, unpublished data). Examination of the transductants derived from the cross donor PAT2235 × recipient PAT2123 showed that 2 out of 100 selected *arg-3129*$^+$ transductants had the donor genotype *aerR101*$^+$ *trp-3117 arg-3129*$^+$, whereas none had the recombinant genotype *aerR101*$^+$ *trp-3117*$^+$ *arg-3129*$^+$. This is consistent with the marker order *aerR101-trp3117-arg-3129*.

Transduction linkage in the 47-min region. The arrangement of the six loci in the 47-min linkage group is shown in Fig. 5. The average cotransduction frequencies between these markers were determined from previous data (33) and from the results of additional transductions. The order of the three *thr* loci, relative to the *sup-1* and *pur-1* loci, was originally based on the results of two-factor crosses (33). Examination of the transductants derived from the cross donor PAT2 × recipient PAT2097 showed that 31 out of 200 selected *thr-1103*$^+$ transductants had the donor genotype *thr-1103*$^+$ *thr-2105*$^+$ *pur-1114*$^+$, whereas none had the recombinant genotype *thr-1103*$^+$ *thr-2105 pur-1114*$^+$. Among 200 selected *pur-1114*$^+$ transductants, 40 had the above donor genotype and none had the above recombinant genotype, whereas 1 transductant had the genotype *thr-1103 thr-2105*$^+$ *pur-1114*$^+$. These observations were consistent with the marker order *thr-1103-thr-2105-pur-1114*, and this has been confirmed by the results of other three-factor crosses involving other alleles of these three loci (Watson, Ph.D. thesis).

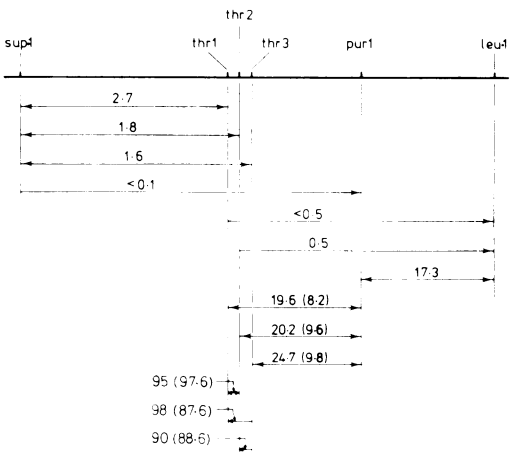

FIG. 5. *Transductional linkage group in the 47-min region. Arrowheads indicate unselected markers. Numbers indicate percentages of cotransduction by F116L or F1083 (in parentheses).*

The position of the *thr-3* locus could not be confirmed by an appropriate three-factor cross. Mutations in the *thr-3* locus confer a requirement for threonine plus homoserine or threonine plus methionine. Thus, the segregation of *thr-3* alleles and *thr-1* (conferring a requirement for threonine) or *thr-2* (conferring a requirement for homoserine) alleles could not be examined (Watson, Ph.D. thesis). The results of a number of two-factor crosses using the smaller phage, F1083, were consistent with the gene order *thr-1-thr-2-thr-3-pur-1*; however, the data were not entirely convincing. Further indirect evidence for this gene order was obtained from the results of transductions between mutants representing each of the *thr* loci (Table 2). It can be seen that the normalized yield of *thr*$^+$ transductants was greater when the *thr-1108* and *thr-3107* mutants were crossed than when the *thr-2106* mutant was crossed with either the *thr-1108* or the *thr-3107* mutant. These results were consistent with the conclusion that the *thr-2* locus is situated between the *thr-1* and *thr-3* loci, as shown in Fig. 5.

Linkage group at 52 min. The linkage relationships between the four markers in the 52-min group were originally determined by Fargie and Holloway (4) from the results of two-factor crosses using phage F116. The cotransduction frequencies by F116L of these markers are summarized in Fig. 6. The marker order *leu-2104-trp-4112-met-3121* was indicated by the results of the cross donor PAT2 × recipient PAT2090, and this was confirmed by analysis of the transductants derived from this cross. Of 400 selected *leu-2104*$^+$ transductants, 156 had the donor genotype *leu-2104*$^+$ *trp-4112*$^+$ *met-3121*$^+$, whereas 4 had the recombinant genotype *leu-2104*$^+$ *trp-4112 met-3121*$^+$. Among the selected *met-3121*$^+$ transductants from this cross, 319 out

TABLE 2. *Transductions between thr mutants of strain PAT*

Recipient	Selected marker	Thr$^+$ transductants[a] per 0.2 ml with donor[b]: PAT2248 (*thr-1108*)	PAT2246 (*thr-2106*)	PAT2247 (*thr-3107*)	PAT2 (*thr$^+$*)
PAT2155	*thr-1108$^+$*	0 (<0.2)	2 (0.8)	9 (3.3)	193
	pur-1118	303	156	172	122
PAT2153	*thr-2106$^+$*	7 (1.1)	0 (<0.2)	0 (<0.2)	236
	pur-1118$^+$	470	370	391	172
PAT2154	*thr-3107$^+$*	19 (2.4)	3 (0.5)	0 (<0.2)	242
	pur-1118$^+$	491	359	325	153

[a] Average number of transductants on duplicate plates. Numbers in parentheses indicate the normalized percent yields of Thr$^+$ transductants as calculated from the formula (A/B) × (D/C) × 100%, where A = number of Thr$^+$ transductants using the mutant donor, B = number of Thr$^+$ transductants using PAT2 as donor, C = number of Pur-1118$^+$ transductants using the mutant donor, and D = number of Pur-1118$^+$ transductants using PAT2 as donor.

[b] The donor strains PAT2246, PAT2247, and PAT2248 are nonlysogenic *pur-1118$^+$* transductants of the recipient strains PAT2153, PAT2154, and PAT2155, respectively.

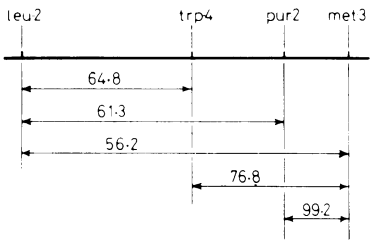

FIG. 6. *Transductional linkage group in the 52-min region. Arrowheads indicate unselected markers. Figures indicate percentages of cotransduction by F116L.*

of 500 had the donor genotype, whereas 10 had the above recombinant genotype.

The results of the cross donor PAT2 × recipient PAT2092 suggested that the *pur-2117* marker was situated between *leu-2104* and *met-3121*. The marker order *leu-2104–pur-2117–met-3121* was confirmed by the fact that 247 out of 500 selected *leu-2104$^+$* transductants had the donor genotype *leu-2104$^+$ pur-2117$^+$ met-3121$^+$*, whereas none had the recombinant genotype *leu-2104$^+$ pur-2117 met-3121$^+$*. Similarly, 229 out of 500 selected *met-3121$^+$* transductants had the donor genotype, whereas none was of the above recombinant genotype. The relative order of *trp-4112* and *pur-2117* with respect to *leu-2104* and *met-3121* has not been confirmed by an appropriate three-factor cross.

Other transductional linkages. Pemberton and Holloway (26) showed that the markers *phe-60* and *his-152* were cotransducible in strain PAO. Similarly, the *his-3* locus of strain PAT was found to be cotransducible with the *phe-5* locus. Of 500 selected *phe-5102$^+$* transductants from the cross F116L.PAT2173 × PAT1068, 133 (26.6%) had also inherited *his-3118*.

Transductional analysis of arginine auxotrophs of strain PAT indicated that the *arg-2* and *arg-6* were very closely linked. These loci are homologous with the *argG* and *argF* loci, respectively, of strain PAO (8; Watson, Ph.D. thesis). Since the *arg-2* mutants respond only to arginine, whereas *arg-6* mutants respond to arginine or citrulline, it was possible to determine the cotransduction of these markers by means of a donor phenotype selection transduction. Of 200 *arg-2119$^+$* transductants from the cross F116L.PAT2117 × PAT2109, selected on citrulline-supplemented MM, 80 (40%) had coinherited *arg-6127*, as indicated by their inability to grow on unsupplemented MM.

The locations of the *his-3*, *arg-2*, and *arg-6* loci on the chromosome have not been precisely determined. Conjugational analysis (Watson, Ph.D. thesis) indicates that *his-3* is probably located between *pro-2* (4 min) and *lys-1* (19 min) and that the *arg-2* and *arg-6* loci are situated between *pur-1* (47 min) and *leu-2* (52 min).

DISCUSSION

The chromosome map of strain PAT is shown in Fig. 7. The locations of the 50 genes on this map have been determined by interrupted conjugation experiments (34) and by analysis of recombinants from plate mating experiments (Watson, Ph.D. thesis). The transductional linkage groups shown on the outer arcs of the circular map have been defined from the data presented in this paper.

Transductional analysis has revealed apparent homology (or at least close linkage) between a number of loci in strains PAT and PAO, and these have similar map locations in both strains (8, 34). The transductional mapping data pre-

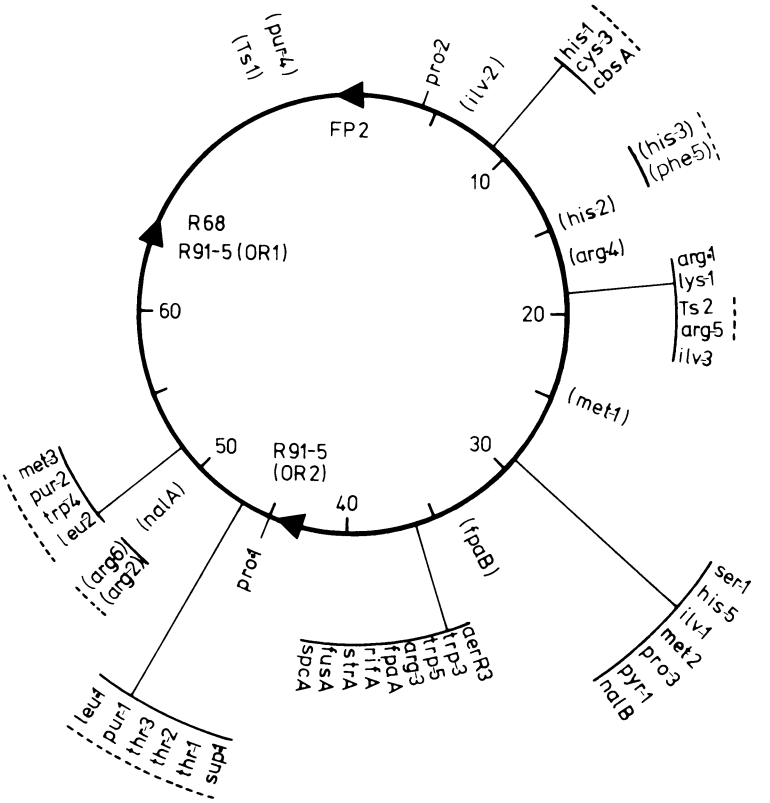

FIG. 7. *Chromosome map of P. aeruginosa PAT. The genes are displayed on a circular linkage group which is calibrated in minutes of transfer time, with the transfer origin of FP2 arbitrarily designated as zero. Arrowheads indicate the sites of the transfer origins of the conjugative plasmids. Markers covered by a dashed arc have not been oriented with respect to the flanking markers. Markers shown in parentheses have only been mapped approximately (Watson, Ph.D. thesis).*

sented in this paper have confirmed the genetic similarity between these strains in particular regions of the chromosome.

Mee and Lee (23) demonstrated cotransduction between the *cys-5605* and *his-5075* markers of strain PAO. The latter marker is situated in the *hisI* region of strain PAO (23, 24), which has been shown to be closely linked to the *hisI* locus of strain PAT (4, 23, 34). The *his-1* locus of strain PAT is cotransducible with the *cys-3* locus and has been mapped at 9 min on the chromosome (34). The *hisI* region of strain PAO is located at 12 min (8).

The markers *his-152* and *phe-60* in strain PAO were shown to be cotransducible by Pemberton and Holloway (26) and to be located at 10 min on the chromosome. The *his-3* and *phe-5* loci of strain PAT are cotransducible and appear to be located on the chromosomal segment between 4 and 19 min. The relationship between these *his* and *phe* markers of strains PAT and PAO has not been examined.

The gene arrangement *arg-1-lys-1-arg-5* at 19 min on the PAT map is identical to the *argH-lys-12-argB* linkage group at 20 min on the PAO chromosome (8). Transduction data indicate that the PAT loci in this linkage group are probably homologous with the respective PAO loci (34; Watson, Ph.D. thesis).

The marker order *ser-3-his-151-ilv-202-pro-70-met-28-pyrB* in the 30-min region of the PAO chromosome has been defined by transduction studies (10, 26). A similar gene arangement was found in the same region of the PAT chromosome, and one additional gene (*nalB*) has been located in this linkage group.

A group of nine genes has been shown to be closely linked in the 36-min region of the PAT chromosome. In strain PAO, the markers *aerR2*, *trp-6*, *argC*, and *str* have also been mapped by transduction (8, 17), and the marker orders are the same in both strains. The five loci which map to the right of *arg-3* in strain PAT (see Fig. 4) have not been ordered unequivocally with

respect to one another. The fact that *fpaA* mutations can be suppressed by certain *str* mutations (32) may prevent confirmation of the order of some of the above loci by means of three-factor crosses.

Three phenotypically distinguishable *thr* mutations were previously shown to be very closely linked in strain PAT (33). The order of the *thr-1* and *thr-2* loci with respect to the *pur-1* locus has been confirmed by three-factor transductions. The position of the *thr-3* locus could not be confirmed by three-factor analysis; however, the available data are consistent with the gene order shown in Fig. 5. In strain PAO, the marker arrangement *thr-1–pur-66–leu-38* has also been defined by transduction (10).

The order of the cotransducible markers in the 52-min region of the PAT chromosome, originally defined by Fargie and Holloway (4), has been examined by means of three-factor crosses. The results of these transductions confirmed that the *pur-2* and *trp-4* loci are situated between *leu-2* and *met-3*. However, the relative order of the former pair of loci remains to be confirmed. A similar arrangement of cotransducible markers has been demonstrated in the late region of the strain PAO chromosome (H. Matsumoto, personal communication).

The limited amount of cotransduction data which has been obtained with phage F1083 supports the conclusion that this phage is smaller than F116L (Chandler, Ph.D. thesis). Although this is not evident from the relative cotransduction frequencies by these phages of very closely linked loci, such as *thr-1*, *thr-2*, and *thr-3*, it is suggested by the relative cotransduction frequencies between the *pur-1* locus and each of the *thr* loci (see Fig. 5).

The fact that the plasmids R68 and R91 can mobilize the PAT chromosome (29, 34) has enabled the mapping of markers in the chromosomal region later than 30 min and has provided evidence for chromosomal circularity in this strain. The length of the chromosomal segment clockwise between the R68 and FP2 transfer origins (see Fig. 7) is not known. Thus, the total length of the *P. aeruginosa* chromosome, in terms of transfer time, cannot be estimated. Resolution of this question will require the isolation of a conjugative plasmid which can mobilize this segment proximally. A continuing search for such plasmids is being made with the aim of increasing the ease of mapping for any region of the *P. aeruginosa* chromosome.

In terms of overall marker arrangement, the chromosome maps of strains PAO and PAT are, as expected, very similar. It is known that these strains are interfertile with both the FP2 and R68.45 sex plasmids. By contrast matings between *P. aeruginosa* and *P. putida* using R68.45 are sterile, although conjugation does occur, as shown by the transfer of the R68.45 plasmid genome. The reasons for this sterility are not known, but it could be due to a lack of nucleotide sequence homology between the two species. Much remains to be learned concerning the comparative arrangements of genes in different species of *Pseudomonas*. It has already been shown (9, 10) that the chromosomal arrangement of genes for biosynthetic pathways in *P. aeruginosa* does not show the clustering so typical of similar genes in the *Enterobacteriaceae*. It remains to be determined if the arrangement of chromosomal genes determining catabolic functions has any significance for the well-known metabolic versatility of this genus. One possibility is that different species will show different gene arrangements of catabolic functions. In this respect it is significant that the gene arrangements of two strains of *P. aeruginosa* isolated from two quite different geographic backgrounds (Australia for PAO, South Africa for PAT) appear to be the same. These results, taken together with our continuing search for plasmids which promote chromosome transfer in and between various species of *Pseudomonas*, highlight the importance of basic mapping data for the understanding of the metabolic versatility and other interesting characteristics of this bacterial genus.

APPENDIX

TABLE 3. *Cotransduction by F116L of markers located in the 9-min region*

Donor	Recipient	Selected marker	Unselected marker	% Cotransduction[a]
PAT2166	PAT2193	*cys-3105*$^+$	*his-1116*	89.0
PAT2193	PAT2166	*his-1116*$^+$	*cys-3105*	88.6
PAT2251	PAT2249	*his-1123*$^+$	*cbsA100*	6.0

[a] Out of 200 to 500 transductants scored.

TABLE 4. *Cotransduction by F116L of markers located in the 19-min region*

Donor	Recipient	Selected marker	Unselected marker	% Cotransduction[a]
PAT2256	PAT2131	*lys-1115*$^+$	*arg-5134*$^+$	1.0
			(Ts) *2104*	1.0
		arg-5134$^+$	*lys-1115*$^+$	1.5
			(Ts) *2104*	89.5
PAT2200	PAT2127	*arg-1130*$^+$	*lys-1115*$^+$	64.6
			ilv-3122	<0.2
		lys-1115$^+$	*arg-1130*$^+$	63.8
			ilv-3122	<0.2
PAT2200	PAT2131	*arg-5134*$^+$	*lys-1115*$^+$	4.0
			ilv-3122	46.0
		lys-1115$^+$	*arg-5134*$^+$	2.8
			ilv-3122	<0.2
PAT2131	PAT2127	*arg-1130*$^+$	*arg-5134*	<0.2
PAT2	PAT2250	(Ts) *2104*$^+$	*lys-1115*$^+$	0.6
		lys-1115$^+$	(Ts) *2104*	1.0
PAT2127	PAT2250	(Ts) *2104*$^+$	*arg-1130*	<0.2
PAT2250	PAT2127	*arg-1130*$^+$	(Ts) *2104*	<0.2
PAT2131	PAT2250	(Ts) *2104*$^+$	*arg-5134*	85.0
PAT2250	PAT2131	*arg-5134*$^+$	(Ts) *2104*	89.0

[a] Out of 200 to 500 transductants scored.

TABLE 5. *Cotransduction by F116L and F1083 of markers located in the 29-min region*

Donor	Recipient	Selected marker	% Cotransduction[a] with unselected marker:						
			ser-1105$^+$	*his-5120*	*ilv-1106*$^+$	*met-2105*$^+$	*pro-3106*$^+$	*pyr-1108*$^+$	*nalB114*
PAT2	PAT2120	*ser-1105*$^+$			2.0	<0.5	<0.5		
		ilv-1106$^+$	2.0			2.5	2.5		
		met-2105$^+$	0.5		1.5		98.0		
		pro-3106$^+$	0.5		4.0	75.0			
PAT2134	PAT2120	*ser-1105*$^+$			5.4	<0.2	<0.2		<0.2
		ilv-1106$^+$	0.6			2.0	1.8		<0.2
		met-2105$^+$	<0.2		3.2		98.0		42.8
		pro-3106$^+$	<0.2		5.0	78.2			41.8
PAT2	PAT2260	*met-2105*$^+$					97.5	84.0	
		pro-3106$^+$				68.0		90.0	
		pyr-1108$^+$				51.0	61.0		
PAT2180[b]	PAT2120[b]	*ser-1105*$^+$		15.4	3.0	<0.2	<0.2		
		ilv-1106$^+$	0.2	77.6		0.6	0.6		
		met-2105$^+$	<0.2	<0.2	0.4		99.0		
		pro-3106$^+$	<0.2	<0.2	0.4	83.0			

[a] Out of 200 to 500 transductants scored.
[b] Transducing phage F1083 used.

TABLE 6. *Cotransduction by F116L of markers located in the 36-min region*

Donor	Recipient	Selected marker	Unselected marker	% Cotransduction[a]
2-2004	PAT2229	*trp-5116*$^+$	*arg-3121*$^+$	74.2
			fpaA101	49.4
		arg-3121$^+$	*trp-5116*$^+$	46.4
			fpaA101	68.4
2-2004	PAT2230	*trp-3117*$^+$	*arg-3121*$^+$	54.6
			fpaA101	36.2
		arg-3121$^+$	*trp-3117*$^+$	42.2
			fpaA101	66.6
PAT2135	PAT2229	*trp-5116*$^+$	*arg-3121*$^+$	74.0
			rifA101	32.2
		arg-3121$^+$	*trp-5116*$^+$	59.0
			rifA101	49.4
PAT2135	PAT2230	*trp-3117*$^+$	*arg-3121*$^+$	65.6
			rifA101	26.6
		arg-3121$^+$	*trp-3117*$^+$	50.0
			rifA101	44.8
PAT2136	PAT2229	*trp-5116*$^+$	*arg-3121*$^+$	58.8
			strA113	13.2
		arg-3121$^+$	*trp-5116*$^+$	46.6
			strA113	30.4
PAT2136	PAT2230	*trp-3117*$^+$	*arg-3121*$^+$	47.2
			strA113	11.0
		arg-3121$^+$	*trp-3117*$^+$	38.0
			strA113	29.6
PAT2262	PAT2189	*trp-3117*$^+$	*arg-3121*$^+$	51.2
			strA116$^+$	7.6
		arg-3121$^+$	*trp-3117*$^+$	59.6
			strA116$^+$	18.3
PAT2163	PAT2229	*trp-5116*$^+$	*arg-3121*$^+$	55.0
			fusA100	8.0
		arg-3121$^+$	*trp-5116*$^+$	36.0
			fusA100	27.6
PAT2163	PAT2230	*trp-3117*$^+$	*arg-3121*$^+$	50.4
			fusA100	5.6
		arg-3121$^+$	*trp-3117*$^+$	35.4
			fusA100	28.6
PAT2137	PAT2229	*trp-5116*$^+$	*arg-3121*$^+$	62.0
			spcA100	6.4
		arg-3121$^+$	*trp-5116*$^+$	42.0
			spcA100	15.6
PAT2137	PAT2230	*trp-3117*$^+$	*arg-3121*$^+$	62.4
			spcA100	3.2
		arg-3121$^+$	*trp-3117*$^+$	36.0
			spcA100	9.6

TABLE 6—*Continued*

Donor	Recipient	Selected marker	Unselected marker	% Cotransduction[a]
PAT1304	PAT2230	*trp-3117*$^+$	*arg-3121*$^+$	59.0
			aerR101	16.0
		arg-3121$^+$	*trp-3117*$^+$	66.0
			aerR101	5.0
PAT2	PAT2124	*trp-3115*$^+$	*arg-3129*$^+$	57.0
			aerR101$^+$	19.0
		arg-3129$^+$	*trp-3115*$^+$	55.0
			aerR101$^+$	5.0
PAT2235	PAT2123	*arg-3129*$^+$	*trp-3117*	86.0
			aerR101$^+$	2.0

[a] Out of 100 to 500 transductants scored.

TABLE 7. *Cotransduction by F116L of markers located in the 47-min region*

Donor	Recipient	Selected marker	Unselected marker	% Cotransduction[a]
PAT2	PAT2155	*thr-1108*$^+$	*pur-1118*$^+$	22.2
		pur-1118$^+$	*thr-1108*$^+$	39.2
PAT2	PAT2153	*thr-2106*$^+$	*pur-1118*$^+$	26.0
		pur-1118$^+$	*thr-2106*$^+$	19.8
PAT2	PAT2154	*thr-3107*$^+$	*pur-1118*$^+$	27.6
		pur-1118$^+$	*thr-3107*$^+$	28.6
PAT2	PAT2070	*pur-1114*$^+$	*leu-1108*$^+$	17.0
		leu-1108$^+$	*pur-1114*$^+$	17.0
PAT2	PAT2097	*thr-1103*$^+$	*thr-2105*$^+$	89.5
			pur-1114$^+$	15.5
			leu-1108$^+$	<0.5
		thr-2105$^+$	*thr-1103*$^+$	95.5
			pur-1114$^+$	12.0
			leu-1108$^+$	0.5
		pur-1114$^+$	*thr-1103*$^+$	20.0
			thr-2105$^+$	20.5
			leu-1108$^+$	18.0
PAT2246	PAT2155	*thr-1108*$^+$	*thr-2106*	96.0
			pur-1118$^+$	7.0
		pur-1118$^+$	*thr-2106*	14.0
			thr-1108$^+$	17.0
PAT2248	PAT2153	*thr-2106*$^+$	*thr-1108*	98.5
			pur-1118$^+$	8.5
		pur-1118$^+$	*thr-1108*	9.0
			thr-2106$^+$	10.5
PAT2246	PAT2154	*thr-3107*$^+$	*thr-2106*	90.0
			pur-1118$^+$	24.0
PAT2248	PAT2154	*thr-3107*$^+$	*thr-1108*	98.0
			pur-1118$^+$	14.0

[a] Out of 200 to 500 transductants scored.

TABLE 8. *Cotransduction by F1083 of markers located in the 47-min region*

Donor	Recipient	Selected marker	Unselected marker	% Cotransduction[a]
PAT2	PAT2155	*thr-1108*$^+$	*pur-1118*$^+$	8.2
PAT2	PAT2153	*thr-2106*$^+$	*pur-1118*$^+$	9.6
PAT2	PAT2154	*thr-3107*$^+$	*pur-1118*$^+$	9.8
PAT2153	PAT2155	*thr-1108*$^+$	*thr-2106*	97.2
PAT2155	PAT2153	*thr-2106*$^+$	*thr-1108*	97.8
PAT2155	PAT2154	*thr-3107*$^+$	*thr-1108*	87.6
PAT2153	PAT2154	*thr-3107*$^+$	*thr-2106*	88.6

[a] Out of 500 transductants scored.

TABLE 9. *Contransduction by F116L of markers located in the 52-min region*

Donor	Recipient	Selected marker	Unselected marker	% Cotransduction[a]
PAT2	PAT2090	*leu-2104*$^+$	*trp-4112*$^+$	52.0
			met-3121$^+$	40.0
		trp-4112$^+$	*leu-2104*$^+$	77.6
			met-3121$^+$	73.8
		met-3121$^+$	*trp-4112*$^+$	79.8
			leu-2104$^+$	65.8
PAT2	PAT2092	*leu-2104*$^+$	*pur-2117*$^+$	64.3
			met-3121$^+$	61.8
		pur-2117$^+$	*leu-2104*$^+$	58.3
			met-3121$^+$	98.3
		met-3121$^+$	*pur-2117*$^+$	100
			leu-2104$^+$	57.3

[a] Out of 400 to 500 transductants scored.

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