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Chromosomal Genetics of *Pseudomonas*

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INTRODUCTION

Over the last 5 years, there has been an increasing interest in the genetics of organisms other than the *Enterobacteriaceae*. The success of the genetic study of *Escherichia coli* has persuaded microbiologists, biochemists, and molecular biologists that solutions to theoretical and applied problems in biology can be successfully approached by understanding genetic phenomena in various microorganisms. Among those organisms which have attracted increasing attention are members of the genus *Pseudomonas*, and the features which enticed earlier workers to the genetic study of this genus remain of interest. They include its notable biochemical diversity, significance for infectious disease, particularly in terms of its resistance to antibiotic therapy, and increasingly the realization that there are genetic phenomena in this genus which are different from those in other bacterial genera. There have been previous reviews on the genetics of *Pseudomonas* (67, 69, 76, 77, 172).

With respect to the biochemical diversity of *Pseudomonas*, considerable attention has been paid to the identification and characterization of plasmids which carry genetic determinants that enable utilization of a wide variety of growth substrates; this aspect has been recently reviewed by Chakrabarty (20). Knowledge of these plasmids and their contribution to the understanding of the genetics and biochemistry of *Pseudomonas* requires a background of intensive and extensive information of the whole genome. For example, it is necessary to be able to differentiate accurately between a function coded for by a chromosomal gene and a function coded for by a plasmid gene.

Much of the sophisticated genetic work on *E. coli* depends on detailed mapping of the chromosome, which has proceeded for over 30 years. There is no reason to believe that progress in genetic studies of other bacteria will be satisfactory without extensive chromosomal mapping data. This article reviews recent studies on the bacterial chromosomal genetics of *Pseudomonas* species.

METHODS OF CHROMOSOMAL ANALYSIS

The three processes of chromosomal transfer, conjugation, transduction, and transformation, are found in *Pseudomonas*, and all have a role in determining genome organization in various

species. In terms of investigating the genetic constitution of the chromosome, conjugation is undoubtedly the most useful. A range of plasmids has been shown to transfer chromosome in *P. aeruginosa*, *P. putida*, and *P. glycinea*. If an overall genetic understanding of the genus *Pseudomonas* is to be obtained, what is needed is plasmids which can promote chromosome transfer in any given species or isolates of this genus. Some of the plasmids which have been previously used for chromosome mobilization studies have a rather limited host range for this ability, but with the demonstration that IncP-1 plasmids have chromosome mobilization ability (Cma) for *Pseudomonas* and the isolation of variants with enhanced Cma (57), the possibility of finding plasmids with wide-host-range Cma becomes, more likely.

Conjugation

In *P. aeruginosa*, FP2 was the plasmid first used for mapping and the basic genetic analysis of this species was carried out with this plasmid. Techniques of recombinant analysis and interrupted mating were shown to be effective in locating the relative position of chromosomal genes. Since then, a variety of other plasmids with Cma has become available. A technique has been developed (38) which enables the identification of Cma plasmids directly in wild-type strains. It has been found that 15 to 30% of hospital isolates of *P. aeruginosa* carry plasmids having Cma, and we refer to this class as FP plasmids. A number of such plasmids have been surveyed for their Cma properties, including the site of origin of chromosome transfer, and although most of them have the same site of origin for chromosome transfer as FP2, some have been isolated with identifiably different properties of chromosome mobilization, such as the ability to transfer "late" markers at much higher frequencies. One plasmid, FP110, has a different origin site (see Fig. 2) and transfers chromosome in the direction opposite that found with FP2 (P. Royle and B. W. Holloway, manuscript in preparation).

One difficulty encountered in using these plasmids for chromosomal analysis of *P. aeruginosa* is that it has not been possible to demonstrate circularity of the strain PAO chromosome as the FP plasmids used have only one predominant site from which the chromosome is transferred. This fact stimulated the search for other plasmids with Cma in *P. aeruginosa* and has led to the examination of various R plasmids. There are now well over 100 R plasmids described for *P. aeruginosa* (93). They have been classified into 10 incompatibility groups. Members of group 1 (or IncP-1) have been extensively studied because of their wide bacterial host range as well as for their ability to promote chromosome transfer (71). It was shown (170; B. W. Holloway, unpublished data) that IncP-1 plasmids have this ability, as do others including R91 (IncP-10). The use of these plasmids was critical to the demonstration of map circularity for the strain PAT chromosome by Watson and Holloway (181). The fact that the IncP-1 plasmids tested did not promote chromosome transfer in strain PAO in turn led to the search for variants of R68, which did have this property, and resulted in the isolation of R68.45 (57, 58). This plasmid can promote chromosome transfer in strain PAO at a frequency higher than that found with FP2 and, furthermore, has a multiplicity of transfer origins, unlike FP plasmids. It displays Cma in all strains of *P. aeruginosa* so far tested. R68.45 contains the full R68 genome together with an additional segment of deoxyribonucleic acid (DNA) inserted near the determinant for kanamycin resistance. This insertion, denoted IS*P*, is ca. 1,800 base pairs long and has been shown to have one *Sma* and two *Pst* restriction endonuclease sites (M. van Montagu and J. Schell, personal communication; G. Reiss, H. J. Burkhardt, and A. Pühler, personal communication; 12a; 88).

R68.45-like plasmids have been derived in the same way from other IncP-1 plasmids, and one has been found in a hospital-isolated strain of *P. aeruginosa*; to date, all those examined possess the IS*P* region (M. Nayudu and B. W. Holloway, unpublished data; Reiss, Burkhardt, and Pühler, personal communication). It is proposed to refer to such plasmids as ECM (enhanced chromosome mobilization) to distinguish them from native Cma plasmids like FP2. The properties of IS*P* in such a plasmid as R68 would include the ability to mobilize chromosome in *P. aeruginosa* strains such as PAO, for which R68 itself has very inefficient Cma. Very likely, IS*P* acts as a recognition site for some chromosomal DNA sequence, and the interaction initiates that sequence of events which we recognize as chromosome transfer. R68 evidently has all the other information necessary for such chromosome transfer, because it displays Cma in *P. aeruginosa* PAT and *P. glycinea* (71, 109, 170, 181). ECM plasmids have also been shown to be active in promoting chromosome transfer in other species of *Pseudomonas*, including *P. putida* (119; H. Dean, A. F. Morgan, and B. W. Holloway, unpublished data) and *P. glycinea* (50).

Considerable effort has been put into the search for a suitable Cma plasmid for *P. putida*. Despite the extensive biochemical knowledge which has been acquired and the variety of degradative plasmids now known for this species, little is known of the genetic organization

of its chromosome. Chakrabarty and Gunsalus (23, 24) derived plasmid pfdm from transducing phage pf16h2, and this is thought to be a bacteriophage-bacterial chromosome hybrid with low-level Cma activity. Subsequently, Shaham et al. (161) showed that the CAM (camphor-utilizing) plasmid could mediate chromosome transfer in *P. putida*, but the frequency of transfer was not adequate for effective chromosomal analysis. Mylroie et al. (137) showed that plasmid K, which is part of the octane-degrading OCT plasmid (21), possessed Cma, but it was too unstable for chromosomal genetic analysis. By isolating recombinant plasmids which contained both plasmids K and XYL (xylene degradation), it was possible to impose selection for the XYL-K plasmid by growth on xylene and thus overcome the problems resulting from instability of the K plasmid. An interrupted-mating technique has been developed, and a preliminary linkage map of *P. putida*, containing some 25 genes, has been published (Fig. 1) (137).

Following the initial demonstration by Martinez and Clarke (119) that R68.45 can promote chromosome transfer in *P. putida*, Dean, Morgan, and Holloway (unpublished data) showed that a variety of ECM plasmids, derived in a manner similar to that for R68.45, show Cma in *P. putida*. Some *P. putida* strains do not display Cma with IncP-1 plasmids. This happens when such strains carry a degradative plasmid of the IncP-2 type. It has been shown (93) that, in both *P. putida* and *P. aeruginosa*, IncP-2 plasmids cause fertility inhibition of IncP-1 plasmids, which markedly reduces both plasmid transfer and IncP-1-promoted host chromosome trans-

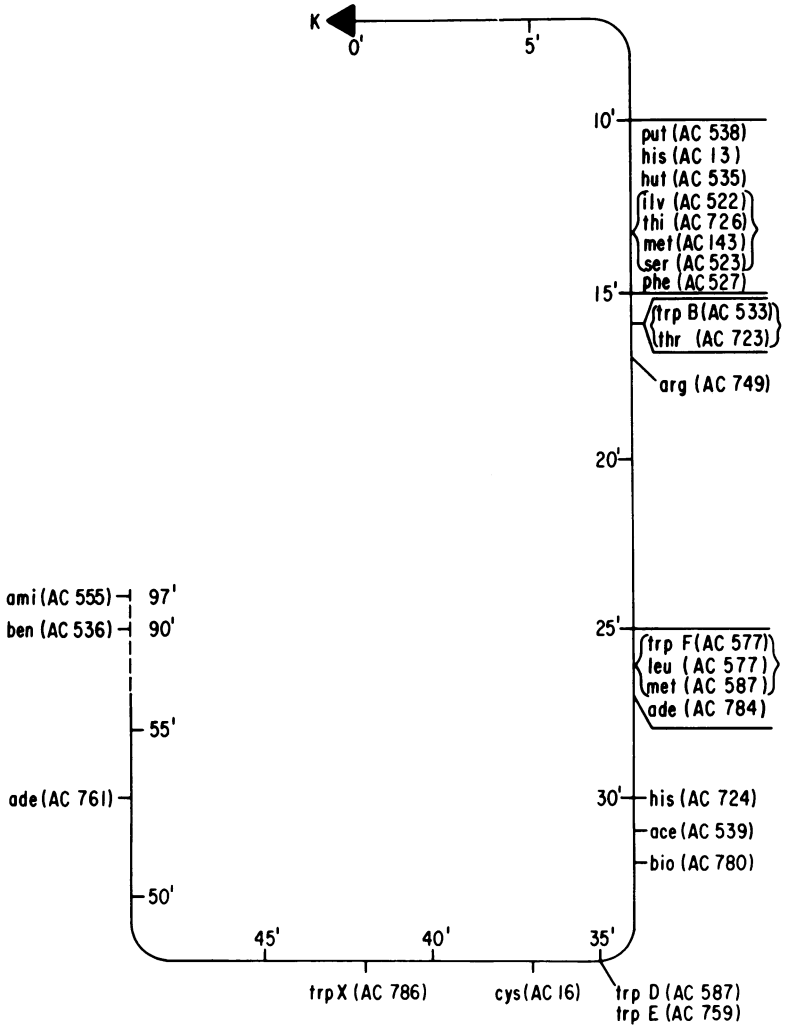

FIG. 1. *Chromosome map of P. putida (137). Designations are as listed in the legend to Fig. 2, together with: ace, acetate utilization; ade, adenine requirement; ben, benzoate utilization; bio, biotin requirement.*

fer. Not all ECM plasmids derived from *P. aeruginosa* show the same Cma characteristics in *P. putida* PRS (ATCC 12633) (H. Dean and A. F. Morgan, unpublished data), the indications being that there are fewer origins with such plasmids in *P. putida* than in *P. aeruginosa* and that the pattern of origins and the efficiency of chromosome transfer are different in these two species.

As described above, FP plasmids with Cma are readily found in *P. aeruginosa*. Many of these plasmids can be "loaded" with transposons (J. Finger and V. Krishnapillai, unpublished data), including FP2, which was the first such plasmid shown to acquire the transposon Tn*1* (144). Acquisition of these transposons confers a potential selective feature such as mercury resistance (Tn*501*) or trimethoprim and streptomycin resistance (Tn*7*), which has enabled selection to be imposed for the transfer of these plasmids to organisms other than *P. aeruginosa*. Most can be transferred to *P. putida*, where they retain Cma, and the lack of coinheritance of unselected markers found with chromosome transfer by these plasmids suggests that relatively small pieces of chromosome are transferred.

In *P. glycinea* it has been shown by Lacy and Leary (109) that IncP-1 plasmids can express Cma, and in a subsequent study Fulbright and Leary (50) claimed that R68 was in fact as effective as R68.45 in this respect. In a series of plate matings, using two- and three-factor crosses, it has been possible to identify the relative chromosomal locations of 11 loci for this species.

ECM plasmids, particularly R68.45, have been shown to promote chromosome transfer in other bacterial genera including *Rhizobium*, *Rhodopseudomonas*, *Azospirillum*, *Agrobacterium*, and *Escherichia* (for a review, see Holloway [71]). This could mean that IS*P* has a nucleotide sequence which can be recognized by a nucleotide sequence (or sequences) found in all these bacteria. It will be of interest to relate IS*P* to those insertion sequences which have already been identified in *E. coli*. We have already found that ECM plasmids can be generated in *E. coli*, indicating that the sequence (or gene product) of IS*P* is not exclusive to *P. aeruginosa* (Nayudu and Holloway, unpublished data). If, as is likely from the data for *P. aeruginosa*, the origin of IS*P* is the bacterial chromosome, it will be of interest to determine what genetic functions it has while still part of the bacterial chromosome.

Phage and Transduction

P. aeruginosa. Bacteriophages which propagate on *P. aeruginosa* are very common, and the properties of many of them have been previously summarized (76). Such phages may be readily isolated from sewage or from hospital isolates of *P. aeruginosa*. Probably all strains of *P. aeruginosa* are lysogenic for at least one phage (76), although it has not always been established whether all such isolates are true lysogens or whether they carry the phage as a persistent infection (pseudolysogeny, the carrier state). The distinction is a fine one and not always a simple matter to distinguish (2). That the nature of phage-host interaction is under genetic control of the host, presumably of the host chromosome, as well as the phage, can be seen from the fact that phages which have been classified as virulent by their behavior with *P. aeruginosa* PAO can be isolated from "purified" bacterial isolates which are presumably lysogenic or more probably pseudolysogenic for the phage (74; B. W. Holloway, V. Krishnapillai and A. F. Morgan, unpublished data). E79 is the best-characterized example of such a phage. It is sufficiently virulent in its effect on PAO to have been used successfully as a contraselective agent in interrupted matings (151) and yet, providing steps are taken to prevent killing of the transductants by phage, E79 is an efficient transducing phage (see below).

With so many phages available, the development of transductional systems for genetic analysis of *P. aeruginosa* has not been difficult. Phages F116L (105) and G101 (78) are generalized transducing phages of 41 and 38 megadaltons, respectively, which both transduce markers at a frequency of 1×10^{-7} to 5×10^{-7} per plaque-forming unit (76). They have been used extensively in mapping of the *P. aeruginosa* chromosome, both for establishing the exact order of closely linked markers and for prototroph reduction tests (44; 124). This test, which functionally performs the same role as meiotic segregation analysis for eucaryotes, may be used to demonstrate that mutations which give rise to similar phenotypes are not closely linked and hence have different genetic bases. The test may be used to assign new mutations to "prototroph reduction groups" that have previously been mapped by conjugation or in the initial genetic analysis of a metabolic pathway. Thus, *P. aeruginosa* PAO mutants isolated on the basis of their inability to utilize mannitol as the sole carbon source have been shown, by F116 transduction, to form four linkage groups (152). When mutations cannot be characterized in this manner, due to close linkage of the genes involved, careful fine-structure analysis using cotransducible outside markers is required before any pronouncement on the genetic basis of a pathway can be made.

Specialized transducing phages have not been

reported for *P. aeruginosa* even though it has been shown that some prophages have a chromosomal location (17, 18, 106). Such phages could prove useful in complementation and dominance studies, assuming that the merozygotes formed are as stable as those formed by λ*dg* transducing particles in *E. coli*. For isolation of specific fragments of the chromosome, however, restriction endonuclease-mediated insertion of chromosomal DNA into suitable plasmid cloning vectors will probably prove a more practical procedure.

Other *Pseudomonas* spp. Other pseudomonads, in particular *P. putida*, have been extensively studied biochemically, but transduction studies have been limited for two reasons. First, phages for other *Pseudomonas* species are much less common than for *P. aeruginosa*, and only in the case of one strain of *P. maltophilia* (134) and one strain of *P. acidovorans* (13) have temperate phages been reported. Second, many laboratories have performed their biochemical studies on *Pseudomonas* isolates found after enrichment of soil or some other natural environment, and these are unlikely to be sensitive to phages developed for genetic analysis of another strain of that particular species. The only *P. putida* phage that has been extensively used for transductional analysis is pf16 (26), which propagates on strain PpG (ATCC 17453), and its host range mutant pf16h2, which can also multiply in *P. putida* PRS (ATCC 12633) (26). The DNA of this phage has a molecular weight of 90 $\times 10^6$, and it has been estimated that it is capable of transducing up to 5% of the *P. putida* chromosome (184). Holloway and van de Putte (79) isolated from sewage a generalized transducing phage, PP1, for *P. putida* PMBL-4BL, but its subsequent use in genetic analysis has not been reported. The same holds for phage M6 of *P. maltophilia* (134). A *P. fluorescens* phage, PX4, which also plates on *P. aeruginosa* PAT, has been shown to be capable of transducing both within strain 14 of *P. fluorescens* and between the two species (145, 146). Recently, a generalized transducing phage, φ12, has been used for genetic analysis of *P. acidovorans* (13).

Interspecific transduction. In general, phages are not suitable for studies involving interspecific gene transfer because of their limited host range and because the exogenote is unable to persist in the recipient without recombination with the recipient genome. Plasmid primes, such as have been developed for *P. aeruginosa* (70; J. Hill and B. W. Holloway, unpublished data), are more suitable for such studies.

A host range mutant of PX4 (146), called Pf20, which plates on *P. putida* PRS has been used to transduce genes involved in tryptophan biosynthesis and benzoate utilization between the two species, although at lower frequencies than found in intraspecies crosses (25). It is difficult to interpret such results in terms of the degree of homology between the relevant genes of the two species, because for stable recombinants to be isolated there is need only for homology between regions flanking the selected gene. R'PA1, which carries the 3- to 4-min region of the *P. aeruginosa* PAO chromosome (70), is highly stable in *P. putida* PRS, and so homology between these two species must be restricted to certain regions of the chromosome (75). A host range mutant of phage M6 of *P. maltophilia*, M6a, is capable of generalized transduction in both its original host and *P. aeruginosa* PAO (134), but its use in interstrain crosses has not been reported. The use of ECM plasmids and R' plasmids seems to be a more efficient procedure for interspecific crosses, and this will be discussed below.

Development of transduction systems. When a temperate phage is used for transduction, it is not usually necessary to prevent killing of the transductants by nontransducing phage particles. However, given the scarcity of temperate phages of pseudomonads other than *P. aeruginosa*, it will probably be necessary to use virulent phages for transduction, and phage killing of transductants will be a problem. The transduction procedure for pf16 uses ultraviolet irradiation of the phage lysate, coupled with the presence of phage antisera to reduce killing, but results can still be variable. A temperature-sensitive mutant of pf16h2 has been used to overcome the problem of transductant killing (178).

Schmieger (159, 160) reported that the *Salmonella typhimurium* phage P22 can be mutated to yield variants capable of transducing chromosomal markers at frequencies up to 100-fold higher than that of the wild-type phage. Morgan (134a) has isolated similar mutants of the virulent *P. aeruginosa* phage E79, designated E79*tv*. Wild-type phage lysates contain transducing particles, but transduction is not normally detectable because the transductants do not survive. Transduction is detectable with phage mutants because a much lower phage input is required to yield a given number of transductants. The recovery of transductants can be increased still further if the recipient cell carries a plasmid of incompatibility group P-2. E79 is one of a number of phages which are female specific as far as these plasmids are concerned. Phage adsorption is normal, but the infection is aborted (90) and cell killing is negligible if the multiplicity of infection is kept below 2 (134a). By using recipients carrying IncP-2

plasmids, it has been possible to show that wild-type E79 is capable of transduction, although at low frequency, as are various other virulent *P. aeruginosa* phages to which IncP-2 plasmids confer resistance.

IncP-2 plasmids can be transferred to many *Pseudomonas* spp. (11), and it may be possible to use this phenomenon to detect virulent phage-mediated transduction in other *Pseudomonas* spp. Unfortunately, IncP-2 plasmids do not confer resistance to pf16 in *P. putida* or PX4 in *P. fluorescens* (A. F. Morgan, unpublished data). However, it should be possible to obtain high-frequency transducing variants of these phages. Of interest in this connection is the isolation of such a mutant for phage MX4 of *Myxococcus xanthus* (16) which, if used at multiplicities of about 100, can transduce chromosomal markers with a frequency of 10^{-2} per recipient. The authors point out that such a phage would be extremely useful in the genetic analysis of markers for which direct selection cannot be applied.

Transformation

For *E. coli*, chromosomal, as opposed to plasmid, transformation can only be detected in a *recB*, *recC*, or *sbcB* (or *A*) recipient because incoming linear DNA is degraded by the *recB* and *recC* endonuclease (35, 143). Equivalent genotypes are not yet available for any *Pseudomonas* spp., but chromosomal transformation has recently been reported for *P. putida* (136) and the phytopathogen *P. solanacearum* (7; C. Boucher, personal communication). In both cases the transformation frequency was about 10^{-6} per recipient cell, and coinheritance of linked markers was demonstrated. For *P. putida*, prior treatment of the recipient cells with Ca^{2+} was required to induce competence, but this was not necessary for *P. solanacearum*. The range of DNA fragment molecular weights able to transform has not been determined.

In *P. aeruginosa* PAO, plasmid transformation of Ca^{2+}-treated recipients has been reported (158, 163), but all attempts to transform chromosomal markers failed. As shearing plasmid DNA reduces transformation frequency but does not yield deletion mutants, unlike the situation for plasmid transformation in *P. putida* (27), it is probable that, as with *E. coli*, linear DNA is degraded. Transformation will probably prove to be more useful for chromosomal analysis of some *Pseudomonas* spp. than for others.

CHROMOSOMAL MAPPING OF *P. AERUGINOSA* PAO

Strain PAO has been more extensively used for genetic work than any other strain of this genus but, despite more loci having been mapped than with other strains, genetic circularity for strain PAO has not yet been demonstrated. Two of the more widely used Cma plasmids, FP2 and FP5 (121), have only one origin of transfer, at a site which has been arbitrarily designated 0 min on the PAO chromosome. These plasmids have been shown to be satisfactory for both plate mating and interrupted matings in the region 0 to 40 min, but for more-distal markers, interrupted matings give too few recombinants for accurate data to be obtained.

R68.45 has been shown (57, 58) to have multiple sites of entry, but in plate matings most recombinants inherit only short donor chromosome fragments which are usually less than 10 min long. However, by the use of double-selection procedures, double recombinants can be isolated by interrupted matings, and these appear after a delay which corresponds to the map distance betwen the two selected markers as measured with FP2 interrupted matings (58). This indicates that R68.45 and FP2 promote chromosome transfer at similar rates, and hence the marker distances obtained with each of these Cma plasmids can be compared, a fact which will be of importance in the quantitation of the strain PAO map.

A variety of other ECM plasmids have been isolated and studied for their mapping characteristics in strain PAO (Nayudu and Holloway, unpublished data), and in general they are very similar to R68.45, some being more stable than R68.45.

A search has been made for additional FP plasmids which have other origins or show increased recovery of markers for the "late" region, i.e., those markers situated later than 40 min on the PAO map. Only one such plasmid has been isolated, FP110, which shows a major site of origin in the 25- to 28-min region and transfers chromosome in the opposite direction to FP2, FP5, and FP39 (Royle and Holloway, in preparation). This has enabled the mapping of a marker, *pur-70*, in the region of the chromosome to the left of the FP2 origin by means of its linkage to *proB* and *ilvB/C*. These three loci also show coinheritance with R68.45 donors. As with FP2, the recovery of markers more distal than *pur-70* from the FP110 origin is very low, and it has not been possible to show linkage of *pur-70* to markers which are very distal in FP2 matings, such as *leu-10* (= *leu-9001*) or *met-9011* (see Fig. 2). This means that at present the length of the chromosome between the markers situated at about 40 to 45 min and the distance between *pur-70* and the FP2 origin cannot be determined quantitatively in terms of minutes

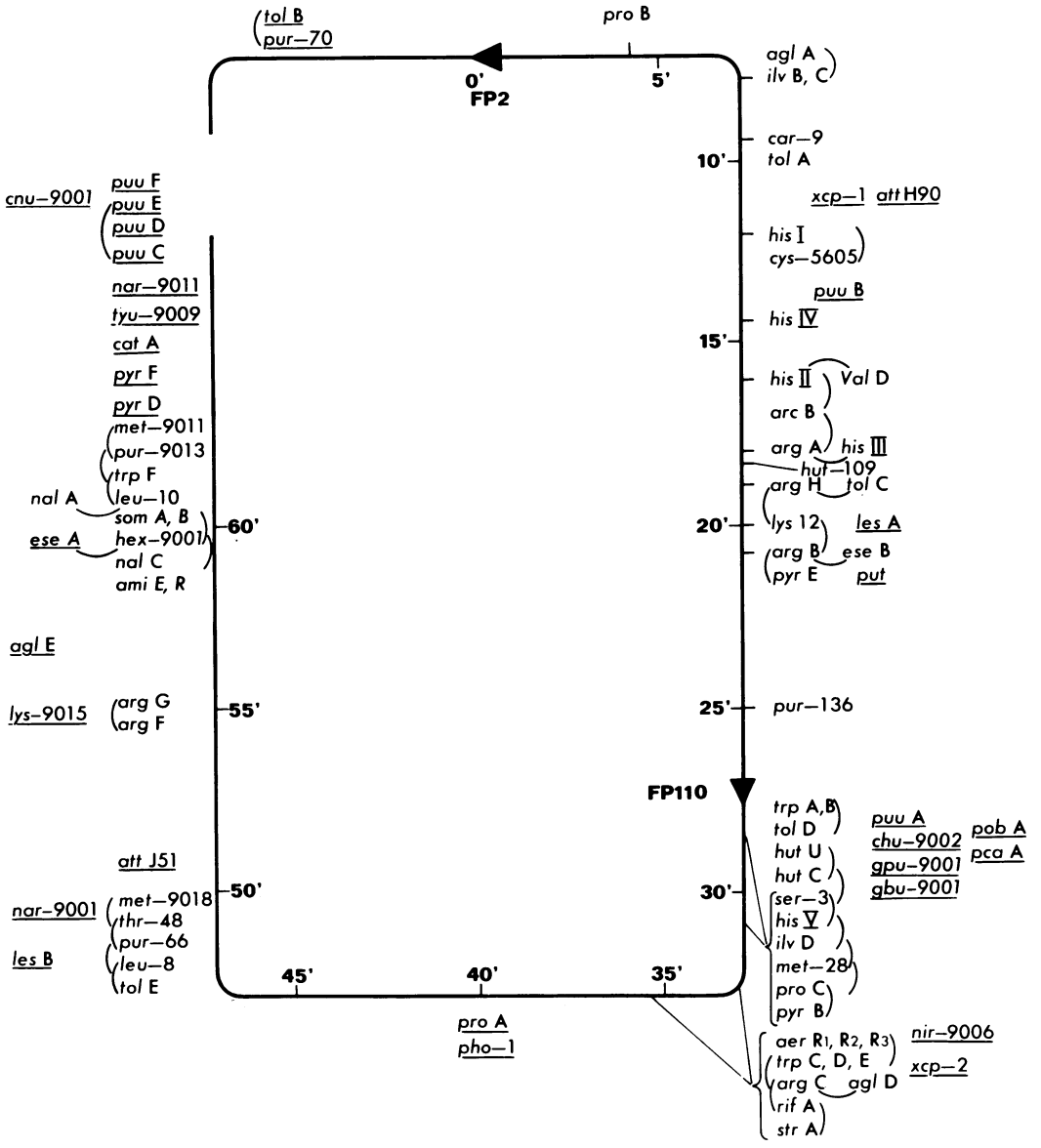

FIG. 2. *Chromosome map of P. aeruginosa PAO. The following symbols have been adopted. (i) Markers whose location is indicated by a bar joining the locus designation to the map were located by interrupted matings, FP2 donors. (ii) Curved lines indicate that the markers are cotransducible with one or more of the phages F116, F116L, G101, and E79tv1. (iii) The marker designation is underlined in those cases in which there is evidence to locate the marker in the area in which the symbol is placed, but the relationships to flanking markers have not been determined. The following marker designations have been used: aer, aeruginocin production; agl, aminoglycoside resistance; ami, amidase; arc, arginine catabolism; arg, arginine requirement; att, prophage attachment site; car, carbamoyl phosphate synthetase; cat, catechol utilization; chu, cholate utilization; cnu, carnosine utilization; cys, cysteine requirement; ese, phage E79 resistance; fpa, p-fluorophenylalanine resistance; fus, fusidic acid hypersensitivity; gbu, guanidinobutyrate utilization; gpu, guanidinoproprionate utilization; hex, hexose utilization; his, histidine requirement; hut, histidine utilization; ilv, isoleucine plus valine; les, lysogenic establishment; leu, leucine requirement; lys, lysine requirement; met, methionine requirement; nal, nalidixic acid; nar, nitrate reductase; nir, nitrite reductase; pca, protocatechuate utilization; phe, phenylalanine requirement; pho, alkaline phosphatase; pob, p-hydroxybenzoate utilization; pro, proline requirement; pur, adenine requirement; put, proline utilization; puu, purine utilization; pyr, uracil requirement; rif, rifampin resistance; ser, serine requirement; som, somatic antigen; spc, spectinomycin resistance; str, streptomycin resistance; sup, suppressor activity; thi, thiamine requirement; thr, threonine requirement; Ts, temperature sensitive for growth; tol, aeruginocin tolerant; trp, tryptophan requirement; tyu, tyrosine utilization; Val, D-valine utilization; xcp, extracellular protease.*

of chromosome transfer by FP2. It has been possible to determine the order of a range of markers in this "late" region, and the details are given in Table 1. Table 1 and Fig. 2 summarize published and unpublished results of the locations of a variety of markers on the strain PAO chromosome. By the use of modified techniques of interrupted mating (59), we have shown that the positions of some markers have required revision relative to earlier published maps. In addition, some markers included in previous maps have been deleted because the strains carrying them have either been lost or no longer show the correct phenotype. Taking as a base the map published by Haas et al. (59), a variety of new markers have been added, and the evidence for the location of each marker is summarized in Table 1. In Table 2 a list of presumably allelic markers is given based on prototrophic reduction tests and other data. These markers have been used in the publications indicated. The map has been constructed with data obtained by plate and interrupted matings using plasmids FP2, FP5, and R68.45. Close linkages have been established by cotransduction with the bacteriophages F116, F116L, G101, and E79*tv*1. In general, the marker designations conform to those used for *E. coli* (1). One deviation concerns markers for which the phenotype involves inability to utilize a particular substrate as the sole carbon or nitrogen source. It is recommended that the three-letter gene symbol consist of two letters indicative of the substrate and the last letter "u" (for utilization).

Although the number of mapped genes is still too few to draw extensive conclusions, there does seem to be a nonrandom distribution of genes involving substrate utilization, and mapping of other examples of this class of mutation will be examined with interest. The size of the region between *leu-10* and the FP2 origin is still unknown. In strain PAT, it was possible to get coinheritance of the markers *pur-4* (corresponding to *pur-70* of PAO) and *leu-2* (corresponding to *leu-10* of PAO) by using R68.45 (181), whereas in PAO, this donor plasmid does not transfer enough chromosome so that coselection can be made for both *leu-10* and *pur-70*. Attempts to use FP2 or FP110 to close the gap have similarly been unsuccessful to date (Royle and Holloway, unpublished data).

A variant form of FP2, possibly analogous to the Hfr form of F, has been described by H. Hermann and E. Gunther (personal communication). It is nontransferable, has a higher efficiency of chromosome transfer, but still transfers chromosome from the same site as does the wild-type FP2 plasmid. This development, together with the addition of transposons to FP plasmids described above, may enable Hfr variants of FP2 and other plasmids to be constructed by selection for integration of an FP plasmid into the chromosome. This could be accomplished by selection of retention of drug resistance with temperature sensitivity plasmids to which a transposon carrying a drug resistance determinant has been added. Watson and Scaife (183) have shown that the IncP-1 plasmid RP4 can acquire an origin of transfer for *E. coli* K-12 by integration.

CHROMOSOMAL MAPPING OF *P. AERUGINOSA* PAT

Genetic studies have been carried out with strain PAT as an alternative to those with strain PAO. It was first shown in this strain by means of transduction studies with F116 that the chromosomal distribution of functionally related biosynthetic markers is different from that established for the *Enterobacteriaceae* (44). Initially, the difficulties of conjugational analysis with strain PAT lay in the fact that it possessed the FP2 plasmid, and PAT(FP2) × PAT(FP2) matings were of very low fertility (66). Stanisich and Holloway (169) showed that nitrogen half-mustards could cure the FP2 plasmid, albeit at low frequency. This led to the construction of a strain, PAT964, which is prototrophic and acts as a recipient in crosses with normal PAT strains containing FP2 or PAT964 derivatives which have acquired FP2. A series of multiply marked strains was built up from PAT964 and these have been used to demonstrate Cma activity of various R plasmids, including R68 and R91 in strain PAT, as well as providing the experimental basis of mapping the PAT chromosome (170, 171).

A detailed conjugational and transductional analysis of strain PAT has been made (181, 182), using plasmids FP2-2 (a variant of FP2 with increased conjugational ability [171]), R91-5 (a derepressed mutant of IncP-10 R91 [30]), and R68 (an IncP-1 plasmid isolated in England). By means of interrupted matings and linkage analysis of recombinants, it has been possible to show that FP2-2 and R68 each mobilize the chromosome from single, but different, transfer origins and that R91-5 mobilizes chromosome from two origins. R68 and R91-5 mobilize the chromosome with a polarity opposite that of FP2-2. The locations of these transfer origins (see Fig. 3) are such that it has not been possible to demonstrate chromosome circularity by means of interrupted-mating experiments with these plasmids. However, with R68.45 which, as in PAO, has multiple origins in PAT, linkage data have been obtained which, together with the available time-of-entry data obtained with

TABLE 1. *Data used for construction of the chromosome map of P. aeruginosa PAO*[a]

Marker	Experimental details of phenotype and location	Reference
aglA	General resistance to aminoglycosides; defective in cytochrome c_{552} and nitrate reductase activity; 50% cotransducible with *ilvB/C*, using F116L	L. E. Bryan, T. I. Nicas, and B. W. Holloway, unpublished data
aglD	Isolated as spontaneous mutant resistant to kanamycin; also resistant to streptomycin, gentamicin, and amikacin; 34% cotransducible with *argC*, using F116L; position relative to flanking markers not determined	C. E. Crowther and B. W. Holloway, unpublished data
aglE	Isolated in a PAO strain carrying R68.45; high-level resistance to aminoglycosides; mapping by FP2 matings and analysis of recombinants	75; B. W. Holloway and C. E. Crowther, unpublished data
aerR1,2,3	R-type aeruginocin determinants; mapping by FP2-mediated conjugation; cotransduction with *trpC,D,E*, using F116	98, 99, 100, 101
amiE *amiR*	Structural (*E*) and regulatory (*R*) genes for amidase; *amiE* and *amiR* shown to be contiguous by transduction deletion mapping with F116; *amiE* located close to *leu-10* by R68.45 matings; from FP5 matings order is probably *argF/G-amiE/R-leu-10*	4, 8; C. E. Crowther and B. W. Holloway, unpublished data; H. Matsumoto, personal communication
arcB	Defect in catabolic ornithine carbamoyltransferase; cotransducible by F116L with *hisII* and *argA*	D. Haas, personal communication
attH90	Insertion site for prophage H90; linkage determined by FP2 matings, zygotic induction, and interrupted matings; located between *car-9* and *argA*	17, 106
attJ51	Insertion site for prophage J51; linkage determined by FP2 matings, zygotic induction, and interrupted matings; located in 50-min region, but linkage to flanking markers not determined	18
catA1	Deficient in catechol 1,2-oxygenase; located distal to *met-9011* and proximal to *tyu-9009* by segregation analysis, using FP5 donors	120
chu-9002	Cannot use choline as sole carbon source; shows 70% linkage in FP5 conjugation with *pyrE*, probably between *pyrE* and *trpA,B*	H. Matsumoto, personal communication
cnu-9001	Inability to use carnosine as either a carbon or a nitrogen source; lacks carnosinase; shows 95% coinheritance with *puuF* in FP5 crosses	H. Matsumoto, personal communication
eseA	Resistance to virulent phage E79; 10% cotransducible with *hex-9001*, using F116	H. Matsumoto, personal communication
eseB	Isolated as resistant to phage E79, with concomitant mutation to *arg-48* which, by prototroph reduction with F116L, is highly linked to *argB*; reversion of *arg-48* to *arg*$^+$ results in concomitant increased sensitivity to E79	B. W. Holloway, unpublished data
gbu-9001	Lacks guanidinobutyrate amidinohydrolase; cannot use guanidinobutyrate as sole nitrogen source; located by FP5 matings between *trpA,B* and *ilv-202*, but relationship to flanking markers not determined	H. Matsumoto, personal communication
gpu-9001	Lacks guanidinoproprionate amidinohydrolase; cannot use guanidinoproprionate as sole nitrogen source; located by FP5 matings between *trpA,B* and *ilv-202*, but relationship to flanking markers not determined	H. Matsumoto, personal communication
hex-9001	Inability to use hexoses; with FP5 crosses shows 98% coinheritance with *met-9011*; probably between *leu-10* and *argF/G*	H. Matsumoto, personal communication
hisIII	Requires histidine for growth; 15% cotransducible with *argA*, using phage F116L; by R68.45 matings, shown to be between *hisII* and *argA*	R. J. Crockett and A. F. Morgan, unpublished data
hisIV	Requires histidine for growth; located by time of entry in FP2 matings; by segregation data, shown to be proximal to *lys-12*, and probably between *hisI* and *hisII*	R. J. Crockett and A. F. Morgan, unpublished data
hisV	Requires histidine; 25% cotransducible with *ser-3* by F116L; by cotransductions with E79*tv*1, the order *ser-3-hisV-ilv-218-met-28* has been demonstrated	B. W. Holloway, unpublished data; R. J. Crockett and A. F. Morgan, unpublished data

TABLE 1—*Continued*

Marker	Experimental details of phenotype and location	Reference
hut-109	Cannot use histidine as sole carbon source; located at 18 min as determined by time of entry only in FP2 matings; relationship to flanking markers not determined	37
hutU	Urocanase deficient; cannot use histidine as sole carbon source; 16% cotransducible with *ser-3*, using F116	37
hutC	Regulatory gene; closely linked to *hutU* by F116 transductions	J. R. Potts (Ph.D. thesis, University of London, London, 1975)
ilvB/C	*ilvB* lacks acetohydroxy acid synthetase, and *ilvC* lacks reductoisomerase; both require isoleucine plus valine for growth; shown to be contiguous by transduction mapping with F116; *ilv-226* (as isolated in PAO substrain 222) shown to be at the same location as *ilvB112*	57, 116; J. M. Carrigan, D. Haas, and B. W. Holloway, unpublished data
ilvD	Requires isoleucine plus valine; lacks dihydroxy acid dehydratase; located between *hisV* and *met-28* by time-of-entry and segregation studies with FP2 matings and by cotransduction with flanking markers, using F116L	116; D. Haas, unpublished data
lesA	Inability to be lysogenized by F116; close to *lys-56* and *argB*; linkage to flanking markers shown by reciprocal crosses with FP2 donors	129
lesB	Inability to be lysogenized by F116; shows Rec$^-$ phenotype; linkage obtained by selection for *leu-8*$^+$ with FP2 matings	129
lys-9105	Requires lysine; in FP5 matings shows 95% coinheritance with *argG*	H. Matsumoto, personal communication
met-9018	Requires methionine; cotransducible, using F116L with *thr-9001* (*thr-48*) and *pur-9012* (*pur-66*), with the order *pur-9012–thr-9001–met-9018*	H. Matsumoto, personal communication
nalA	High-level resistance to nalidixic acid; 1% cotransduction with *leu-10*; <1% cotransduction with *hex-9001* (F116L used)	57, 58; D. Haas, personal communication
nalC	High-level resistance to nalidixic acid; 23 to 32% cotransduction with *hex-9001*; <1% cotransduction with *leu-10* (F116L used)	59; D. Haas, personal communication
nar-9001	Blocked in the conversion of nitrate to nitrite; shows 80% linkage to *leu-38* in matings with FP5 donors; could be *narB* or *narC*	83; H. Matsumoto, personal communication
nar-9001	Blocked in the conversion of nitrate to nitrite; located from segregation data obtained with FP5 donors; shows linkage to *tyu-9009* and *puuC,D,E*; may correspond to *narA* or *narD*	83, 120; H. Matsumoto, personal communication
nir-9001	Lacks dissimilatory nitrite reductase activity; shows linkage to *tyu-9001* and *met-9011* in matings with FP5 donors	H. Matsumoto, personal communication
nir-9006	Lacks dissimilatory nitrite reductase activity; may correspond to *nirB*; located by segregation data with FP5 donors	83, 120
pcaA	Lacks protocatechuate oxidase; located by FP5 matings between *ilv-202* and *trpA,B*	H. Matsumoto, personal communication
pho-1	Deficient in alkaline phosphatase; shows 96% coinheritance when *proA82* is selected in crosses with R68.45	B. Wretlind, personal communication
pobA	Lacks *p*-hydroxybenzoate hydroxylase; located by FP5 matings between *ilv-202* and *trpA,B*	H. Matsumoto, personal communication
proA	Defect in glutamate 5-phosphotransferase; mapped, by FP2 and R68.45 matings, between *trpC,D,E* and *leu-38*	R. V. Krishna and T. Leisinger, personal communication
proB	Defect in glutamyl 5-phosphate reductase; location identical to that of *pro-4* on Loutit map; mapping by interrupted mating with FP2 and segregation data with R68.45 donors; situated between *ilvB/C* and FP2 origin	114; R. V. Krishna and T. Leisinger, personal communication; J. M. Carrigan, D. Haas, and B. W. Holloway, in preparation
proC	Lacks ability to convert Δ-pyrroline 5-carboxylate to proline; by F116 transduction with flanking markers location shown to be *ilv-202–met-28–proC*	151, 179
pur-70	Requires adenine for growth; located between *met-9011* and the FP2 origin; linkage in FP110 crosses to *ilv-219*	P. L. Royle and B. W. Holloway, unpublished data

TABLE 1—*Continued*

Marker	Experimental details of phenotype and location	Reference
put	Cannot use proline as sole carbon source; enzyme deficiency not determined; location to other markers not determined; located, by time of entry, at ca. 22 min with FP2 donor	37
puuA	Lacks adenine deaminase; mapped by recombinant analysis with FP5; 96% recovery of *puuA* marker when double selection imposed for *trpA*$^+$ *ilv-202*$^+$ in FP5 matings	120
puuB	Lacks guanine deaminase; linkage data derived from recombination analysis with FP5 donors shows location to be between *hisI* and *hisII*	120
puuC	Lacks xanthine dehydrogenase; FP5 matings indicate a location later than 50 min but position not accurately determined relative to flanking markers; closely linked by G101 transduction to *puuD* and *puuE*, in the relative order *puuC-puuD-puuE*	120
puuD	Lacks uricase; linkage determination as for *puuC*	120
puuE	Lacks allantoinase; linkage determination as for *puuC*	120
puuF	Lacks allantoicase; not cotransducible with *puuC,D,E* cluster; analysis of recombinants from FP5 crosses suggests the order *tyu-nar-puuD-puuF*	120
pyrB	Lacks aspartate transcarbamylase; by cotransduction with F116 *met-28* shown to be between *ilv-202* and *pyrB*	87
pyrD	Requires uracil for growth; lacks dihydroorotic acid dehydrogenase; by three-factor R68.45 crosses, order shown to be *leu-9001-met-9011-pyrD*	R. J. Crockett and A. F. Morgan, unpublished data
pyrE	Lacks orotidylic acid pyrophosphorylase; located by time-of-entry data with FP114 and F116L cotransduction with *argB* (65%); not cotransducible with *lys-12*	87; P. L. Royle, personal communication
pyrF	Requires uracil for growth; lacks orotidine monophosphate pyrophosphorylase; by three-factor R68.45 crosses, order shown to be *leu-9001-met-9011-pyrF*, with *pyrF* probably between *met-9011* and *pyrD*	R. J. Crockett and A. F. Morgan, unpublished data
rifA	Resistance to rifampin; cotransducible with *trpC, trpD,* and *argC*; shown by transduction to be between *strA* and *argC*	C. E. Crowther and B. W. Holloway, unpublished data
ser-3	Requires serine; 25% cotransducible with *hisV*	B. W. Holloway, unpublished data
somA	Somatic antigen marker, identified by agglutination techniques; 15% cotransducible with *leu-9001* and 2% cotransducible with *hex-9001*, using F116	122; H. Matsumoto, personal communication
somB	Somatic antigen marker, identified by agglutination technique; 12% cotransducible with *leu-9001* and 20% cotransducible with *hex-9001*, using F116	H. Matsumoto, personal communication
thr-48	Requires threonine; 29% cotransducible with *pur-66*, using F116	151
tolB	Isolated as tolerant to aeruginocin AR41, with concomitant mutation to purine requirement (*pur-70*); *tolB* and *pur-70* are cotransducible with F116L	H. Rossiter and B. W. Holloway, unpublished data
tolC	Isolated as tolerant to aeruginocin AR41, with concomitant mutation to arginine requirement (*arg-11*); *arg-11* shown by prototrophic reduction with F116L to be very closely linked to *argH*; *tolC* is 69% cotransducible with *arg-11*, using F116L	H. Rossiter and B. W. Holloway, unpublished data
tolD	Isolated as tolerant to aeruginocin AR41, with concomitant mutation to tryptophan requirement (*trp-62*) (not satisfied by indole); *trp-62* shown by prototrophic reduction with F116L to be very closely linked to *trpA* and *trpB*; *tolD* is 100% cotransducible with *trp-62*	80; H. Rossiter and B. W. Holloway, unpublished data
tolE	Isolated as tolerant to aeruginocin AR41; also shows Les$^-$ phenotype (reduced ability to establish lysogeny with certain temperate phages); shows 100% linkage to *leu-8* in conjugation with FP2 donors	J. F. Dodge and B. W. Holloway, unpublished data

TABLE 1—*Continued*

Marker	Experimental details of phenotype and location	Reference
trpA	Requires tryptophan or indole for growth; lacks tryptophan synthase (α activity); >95% cotransducible with *trpB*, using F116L; formerly *trpE*	15; B. W. Holloway, unpublished data
trpB	Requires tryptophan for growth; lacks tryptophan synthase (β activity); located by time of entry in FP2 matings; formerly *trpF*	15, 59
trpC	Requires tryptophan or indole for growth; lacks indole-glycerol phosphate synthase; 32% cotransducible with *argC* and 18% cotransducible with *rifA*, using F116L; formerly *trpD*	15; B. W. Holloway, unpublished data
trpD	Requires tryptophan or indole for growth; lacks anthranilate phosphoribosyltransferase; 21% cotransducible with *argC* and 13% cotransducible with *rifA*, using F116L; formerly *trpB*	15; B. W. Holloway, unpublished data
trpE	Requires tryptophan, anthranilate, or indole for growth; lacks anthranilate synthase; closely linked to *trpC* and *trpD* by prototroph reduction tests with F116L, but no cotransduction detected with *argC* or *rifA*; formerly *trpA*	15; B. W. Holloway, unpublished data
trpF	Requires tryptophan or indole for growth; lacks phosphoribosyl anthranilate isomerase; 60% cotransducible with *pur-9103* and 40% cotransducible with *leu-10*, using F116L; formerly *trpB*	15; P. L. Royle, unpublished data
tyu-9009	Unable to grow on tyrosine as sole carbon source; lacks homogentisicase; between *nar-9011* and *catA1*; linkage data obtained from segregation of *tyu* and flanking markers in crosses with FP5	120
ValD	Cannot use DL-valine, DL-alanine, or L-proline as sole carbon source and incapable of induction of D-amino-acid oxidase; 30 to 44% cotransducible, using F116L, with *hisII*	115
xcp-1	Lack of extracellular protease probably due to decreased ability to release extracellular protein; 72% coinheritance with *ilvB/C* and 65% coinheritance with *hisII* in crosses with R68.45, so probably located between *ilvB/C* and *hisII*	187, 188; B. Wretlind, personal communication
xcp-2	Does not produce an extracellular protease, probably due to mutation effects on a common regulatory mechanism for extracellular protein; 93% coinheritance when *trp-6* is selected in crosses with R68.45	188; B. Wretlind, personal communication

[a] Based on the map published by Haas et al. (59) and data for the location of markers cited in that map are given in that reference.

FP2-2, R68, and R91-5, have conclusively demonstrated that the chromosomal genetic map of *P. aeruginosa* PAT is circular.

The use of transduction to demonstrate linkage of other markers to those markers whose position has been established by conjugation analysis has resulted in the construction of a linkage map of *P. aeruginosa* PAT which includes some 50 markers (182) (Fig. 3). One feature of this linkage analysis was the demonstration of a cluster of markers affecting threonine biosynthesis. Transduction analysis shows them to be very closely linked, and they may be contiguous, this reasoning being based on the estimated molecular weight of the *P. aeruginosa* chromosome (2.1×10^9) (150), the molecular weight of the transducing phage, and the frequency of cotransduction. These three loci may represent part of a regulatory unit concerned with threonine biosynthesis.

Another region of interest is that involving the streptomycin resistance, fusidic acid hypersensitivity, and spectinomycin resistance loci, all of which show close linkage and may code for ribosomal proteins. The close linkage of the rifampin resistance locus, which in *E. coli* and *P. aeruginosa* has been shown to code for the beta subunit of ribonucleic acid polymerase (164, 165), to this region of putative ribosomal protein genes further suggests that this may be a region of the chromosome whose gene products are involved in macromolecular synthesis.

A comparison of the PAO and PAT maps shows no essential differences between the two strains, and, indeed, where gene identification has been possible in terms of gene products, there is an extremely close similarity. Although restriction enzyme differences between PAO and PAT make direct mapping comparisons difficult, the strains are certainly interfertile, as shown

TABLE 2. *Chromosome map of P. aeruginosa PAO*[a]

Marker symbol on map (Fig. 2)	Closely linked (allelic) markers
argA	*arg-163* (151)
argB	*arg-1* (151); *arg-18* (151)
argC	*arg-6* (114); *arg-54* (151)
argH	*arg-32* (59, 151)
car-9	*arg-160* (151); *arg-161* (151); *car-160* (59); *car-161* (59)
cys-5605	*cys-61* (151)
hisI	*his-5039* (125); *his-5075* (124, 125); *his-9004* (120)
hisII	*his-4* (57, 59); *his-12* (59); *his-07* (115, 124); *his-35* (115, 124); *his-68* (115, 124); *his-151* (59, 151); *his-154* (151); *his-9010* (120); *his-9011* (120)
ilvB/C	*ilv-219* (57, 59); *ilv-226* (57, 59); *ilv-261* (151)
ilvD	*ilv-202* (57, 59, 151); *ilv-9012* (120)
leu-8	*leu-1* (114); *leu-38* (57, 59); *leu-41* (151); *leu-9006* (120); *leu-9008* (120)
leu-10	*leu-9001* (120)
lys-12	*lys-2* (114); *lys-53* (151); *lys-56* (151); *lys-58* (59); *lys-60* (59); *lys-61* (59); *lys-9006* (120)
met-28	*met-11* (151)
met-9011	*met-9020* (120)
nalC	*nal-7* (59); *nal-8* (59); *nal-12* (59); *nal-13* (59)
proA	*pro-73* (151); *pro-82* (59)
proB	*pro-4* (114); *pro-64* (59); *pro-71* (151)
proC	*pro-70* (151)
pur-66	*ade-5* (114); *pur-9012* (120)
strA	*str-1* (57, 59); *str-2* (58); *str-7* (57, 59); *str-43* (59)
thr-48	*thr-1* (172); *thr-9001* (120)
trpA,B	*trp-150* (151); *trp-9025* (120); *trp-9027* (120)
trpC,D,E	*trp-6* (57, 58); *trp-54* (57)
tyu-9009	*tyu-9017* (120); *tyu-9019* (120)

[a] Relationship of other published markers to markers referred to in Fig. 2. Numbers in parentheses are references.

initially with FP2 (67) and also more recently with R68.45 (57). In view of the interest in gene relationships between different species of *Pseudomonas* (discussed elsewhere in this article), it is significant that the gene arrangements of these two strains of *P. aeruginosa*, PAO and PAT, which come from quite separate geographical origins (Australia and South Africa, respectively), appear to be the same. Data of this type will undoubtedly be more interesting when more distantly related species or strains are compared, and they can only be obtained after construction of detailed chromosomal maps. Linkage data, although difficult and time consuming to obtain, are absolutely necessary for understanding the biological and biochemical characteristics and the evolutionary origins of such a diverse genus as *Pseudomonas*.

INTERSPECIFIC AND INTERGENERIC CROSSES

The properties of ECM plasmids derived from IncP-1 plasmids, in particular their ability to move freely between different species of *Pseudomonas* and other bacterial genera, provide means by which transfer of genetic material between different species of *Pseudomonas* and even between *Pseudomonas* and other bacterial genera may be achieved. In many cases involving the transfer and expression of heterologous DNA, selection can be made for expression of a particular phenotype, for example, the conversion of an auxotrophic phenotype to a prototrophic one, but in most cases it is not possible to be certain that the incoming allele of the marker in question is expressing itself, as some form of suppressor gene activity could give the same phenotypic result. When any alteration of recipient phenotype does take place in this type of genetic exchange, we shall use the term complementation. R′ plasmids, such as R′PA1 (70), are particularly suitable for this type of experiment, and it has been demonstrated (75) that specific genes of *P. aeruginosa* can be transferred to *P. putida* and can complement mutant alleles in that recipient. By using R′PA1, which covers the chromosomal segment including *argA*, *argH*, *lys-12*, and *argB*, it was shown, for example, that transfer of R′PA1 to a *P. putida* mutant deficient in argininosuccinase (*argH*) complemented the deficiency so that the *P. putida* recipient containing R′PA1 was prototrophic. It was shown that *P. putida argH* containing R′PA1 could synthesize argininosuccinase, but it was not demonstrated that this was the enzyme produced by *P. aeruginosa*. This basic technique has considerable promise for

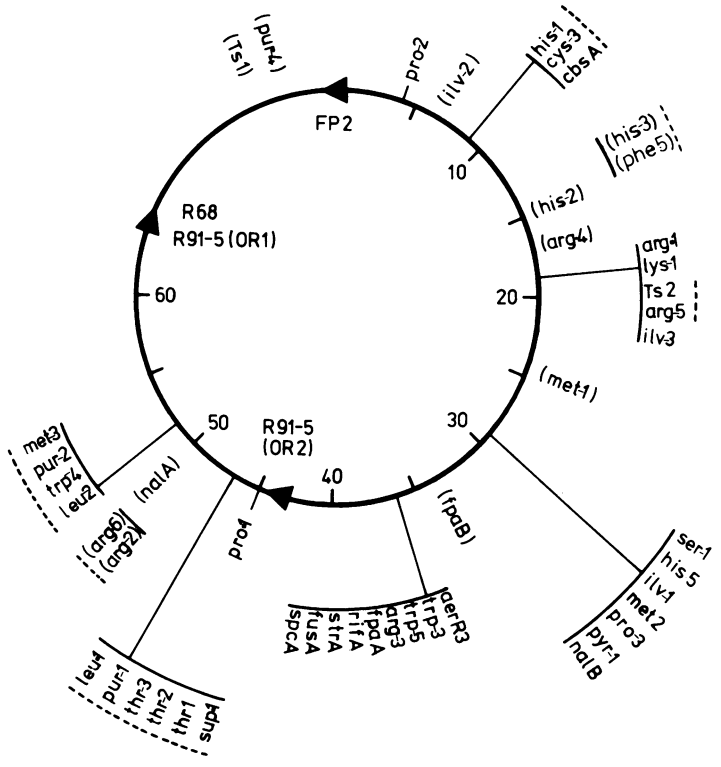

FIG. 3. *Chromosome map of P. aeruginosa PAT (181). Designations are as listed in the legend to Fig. 2.*

studying expression of *Pseudomonas* genetic material in various heterospecific backgrounds and should be of value in comparing those species of the genus in which there are pronounced biochemical and other differences.

The R′ plasmids derived from R68.45 have also been used to study the transfer of genetic material between unrelated genera. Hedges et al. (62) isolated an R′ plasmid from *P. aeruginosa* which contained the chromosomal *trpA,B* genes coding for the alpha and beta chains of tryptophan synthase. This was transferred into *E. coli*, where it was found that, if these hybrids were provided with anthranilate in place of tryptophan, the generation time was more than 10 h. It was possible to obtain plasmid mutants which were constitutive for tryptophan synthase; this mutation was very likely located on plasmid-carried chromosomal material. It was concluded that regulation of the *trpA,B* genes in the *E. coli* cytoplasm was similar to that which occurred in *P. aeruginosa* rather than that demonstrated in *E. coli*. It is clear that there is an impediment to the expression of certain *Pseudomonas* genes in *E. coli*.

These observations were extended by Hedges and Jacob (61), who obtained plasmids derived from R68.45 containing, respectively, *trpE*$^+$, *hisB*$^+$, *argE*$^+$, *guaA*$^+$, or *argG*$^+$, which could each be transferred to *E. coli* and there complement the appropriate auxotrophic lesion. For these transfers, no enzymatic determinations of the gene products involved were made. Domaradskij and his colleagues (41, 42, 48, 133) have also studied the ability of IncP-1 plasmids to transfer genetic material between *E. coli* and *P. aeruginosa* and have demonstrated that a plasmid-bacterial chromosome hybrid is formed.

Nagahari et al. (139) constructed an RP4-*trp* plasmid consisting of RP4 DNA and the complete tryptophan operon of *E. coli* obtained from the bacteriophage $\lambda trpE,A^{60-3}$ in vitro. This plasmid was transferred from *E. coli* to *P. aeruginosa*, and the activities of anthranilate synthase and the beta unit of tryptophan synthase were measured. It was found that the levels of both enzymes in a *P. aeruginosa* strain carrying the RP4-*trp* plasmid were markedly elevated compared with the enzyme levels found in the prototrophic *P. aeruginosa* strain with only chromosomally located *trp* genes. Furthermore, the same enzyme activities were found in both nonrepressed and repressed conditions.

Expression of genes transferred between *P.*

aeruginosa and *E. coli* has also been studied with plasmid-coded genes, in particular those carried by the TOL plasmid. Benson and Shapiro (3) produced a hybrid TOL plasmid containing transposon Tn*401*, which codes for carbenicillin resistance, and transferred this hybrid plasmid from *P. aeruginosa* to *E. coli*. Whereas the replicative and conjugative functions of the plasmids are expressed by both hosts, as is the production of β-lactamase, the ability to grow on *m*-toluate is expressed only by *P. aeruginosa* or *P. putida* and not by *E. coli*. Similar results were obtained by Jacoby et al. (92) using a hybrid between TOL and RP4, and it was found that *E. coli* strains carrying the RP4-TOL plasmid formed colonies on media with toluene, xylene, or *p*-xylene as the sole carbon source only after 4 to 5 days. Growth did not occur with benzoate or *m*- or *p*-toluate. Nakazawa et al. (141) also obtained a recombinant between RP4 and a thermosensitive TOL plasmid and found that, even under conditions of induction, the synthesis of *m*-pyrocatechase in *E. coli* was about 1% of the figure obtained for *P. putida*. These are other examples of an impediment to the expression of *Pseudomonas* genes in *E. coli*.

By contrast, the transfer of *E. coli* genes to *Pseudomonas* does not seem to show any such an impediment for expression. Mergeay and Gerits (128) have shown that various F′ plasmids of *E. coli* can be transferred into *P. fluorescens*, where they can complement a wide range of auxotrophs. Mutants with requirements for isoleucine, leucine, methionine, arginine, and histidine were complemented well, but tryptophan mutants were at best complemented only poorly. By means of these plasmids it was possible to identify the enzymes involved in metabolic blocks in *P. fluorescens*. In a subsequent study, Mergeay et al. (127) showed that *argE* (acetylornithinase) and *argH* (argininosuccinase) gene products could be detected in *P. fluorescens* carrying the *E. coli* F′ plasmids with these particular genes. The *E. coli* genes are not repressible by arginine in *P. fluorescens*, as the transregulatory gene of the arginine regulon is not carried by the plasmid. The specific activities of the *argE* and *argH* enzymes of *E. coli* expressed in *P. fluorescens* are similar to the values found for wild-type *E. coli* cells synthesizing arginine endogenously. These results suggest that there is no serious limitation to the expression of *E. coli* genes in *P. fluorescens*. It has recently been shown (103) that a plasmid, pRD1, containing the *nif* genes of *Klebsiella* can be transferred to *Bacillus subtilis* and there express the nitrogen fixation phenotype. Collectively, these results suggest that soil organisms such as *Pseudomonas* and *Bacillus* are much more adept at expressing information from DNA which they acquire from a range of sources compared to an enteric organism such as *E. coli* with a very restricted and definite ecological niche.

R-prime derivatives have been obtained by using R68.45 in *Rhizobium leguminosarum*. When such R primes carrying different tryptophan genes were transferred to *P. aeruginosa*, they readily suppressed *trpA*, *-B*, *-C*, *-D*, *-E* and *-F* mutants of that strain. However, when the R-prime plasmids were transferred to *E. coli trp* strains, they failed to suppress any *trp* mutants, although it was possible to isolate a plasmid mutant that could express *Rhizobium* genes in *E. coli* (96).

Clearly, much remains to be done in the transfer and expression of interspecific and intergeneric genetic material, but should it become clear from this work that species of *Pseudomonas* are better at expressing foreign genes than is *E. coli*, there may be a case for using *Pseudomonas* species, in particular the nonpathogenic varieties, for elaboration of specific gene products in in vitro genetic engineering experiments. Organisms such as *P. fluorescens* have much to commend them for such experiments in terms of nonpathogenicity and ease with which they will grow on a variety of substrates. What they lack is a well-characterized genetic system as well as suitable plasmid vectors. A beginning has been made in looking for vectors that can be used in such species by Nagahari and Sakaguchi (139), who have shown that the RS1010 plasmid derived from an *E. coli* plasmid can be introduced by transformation into *P. putida* and *P. aeruginosa*, where it has potential use as a vector. This plasmid has a multiple copy number of 43 per chromosome equivalent in *P. putida* cells, it is nonconjugative, and has only one DNA site susceptible to *Eco*RI and *Hpa*I endonucleases and three sites susceptible to *Hin*cII restriction endonuclease. One difficulty with this plasmid is its reduced stability in some *Pseudomonas* strains.

GENETIC REGULATION

Introduction

Detailed reviews on regulation of gene expression in bacteria, including *Pseudomonas*, have been recently prepared (31–33). We shall consider three aspects of particular interest in *Pseudomonas*: gene arrangement, the amidase system, and suppression.

Arrangement of genes in *Pseudomonas*. The literature on gene arrangement up to 1975 has been extensively reviewed (34, 36, 69, 184). It was recognized early that the genes for biosynthetic pathways were nonclustered in *P.*

aeruginosa (44), which is quite unlike the distribution in *E. coli* or *S. typhimurium* (1, 157). For example, the histidine genes form a contiguous sequence of nine structural genes in enteric bacteria, whereas at least five unlinked loci are involved in *P. aeruginosa* (125; R. J. Crockett and A. F. Morgan, unpublished data). Where mapping has been carried out in *P. putida*, this lack of clustering of biosynthetic pathway genes also seems to be true (137). By contrast, *P. putida* is unique in possessing marked clustering for those genes controlling the dissimilatory pathways. This was first demonstrated by pf16h2 transduction for the mandelate (26) and β-ketoadipate pathways (185).

Particular attention has been paid to the arrangement of genes involved in the β-ketoadipate pathways (166). Cotransductional linkage in two-factor crosses with phage F116 in *P. aeruginosa* has established two transductional clusters controlling this pathway, with only one of the genes, *pcaA* (specifying protocatechuate oxygenase), not being linked to either cluster (102, 156). A similar analysis with phage pf16h2 in *P. putida* has also identified two transductional clusters, but in this case *pcaA* was found to be linked to one of the clusters (185). A striking feature of the genetic organization of the pathway in these two species was the separation of the mandelate genes *mdlC* and *mdlB* by *catC*, *catB*, and *ben* in *P. aeruginosa*, whereas the mandelate genes are all linked to each other in *P. putida*. Otherwise, all the genes of this pathway map at corresponding places on the respective genomes. Stanier and Ornston (166) have speculated that the genes governing the central reactions of this pathway anteceded the evolutionary separation of these species, with subsequent independent development of the genes of the mandelate pathways (166). A recent genetic analysis by FP2-mediated conjugational and F116L- or G101-mediated transductional crosses has mapped six genes of the arginine biosynthetic pathways and the gene controlling carbamoylphosphate synthase in *P. aeruginosa* PAO (59). The *argA,argH,argB* loci map within about 3 min of each other on the chromosome, and *argF,argG* are cotransducible but not contiguous. These two genetic clusters are unlinked to either *car* or *argG*. Also in strain PAO, four unlinked groups of genes which control the catabolism of mannitol and fructose have been identified by two-factor crosses with F116 (6, 152). In *P. putida*, five genes specifying glucose dissimilation have been shown by pf16h2 to be cotransducible (176). The inferences drawn about the 90° differences in orientation of glucose utilization genes on the chromosome of *E. coli* K-12, such as their separation after two sequential duplications of an ancestral genome (155), may not be expected to apply in *P. putida* because of its unique pathway of glucose assimilation, which is quite unlike that of most other bacteria (176). In any case, this extends the concept of genetic clustering of genes controlling catabolic pathways previously established in *P. putida* to those affecting the degradation of *p*-hydroxybenzoate, quinate, shikimate, benzoate, mandelate phenylacetate, nicotinate, phenylalanine, tyrosine, and histidine (184). It is remarkable that, on the basis of pf16h2 transductional analyses, 21 structural genes believed to form 14 regulatory units controlling the dissimilation of these substrates map within about 10 to 15% of the bacterial chromosome (110). However, this important generalization with respect to clustering of these genes on the chromosome has to be confirmed in future genetic analyses by demonstration of their linkage to other chromosomal markers. At present there are insufficient linkage data to exclude the possibility that certain catabolic genes are on very stable plasmids rather than on chromosomes (which would indeed support the speculations regarding the value of such clustering, e.g., the suggestion of the potential for genetic transfer [110]). It has recently been shown that nicotine-degrading genes are plasmid borne in *P. convexa* (174), whereas the equivalent genes are believed to be chromosomal in *P. putida* (110). Although the comparable genes in *P. putida* and *P. convexa* have not been shown to be genetically homologous, one interpretation is that they are on the same plasmid, but differential plasmid stability is manifested in the different hosts. The initial supposition of plasmid-borne genes in *P. putida* was based primarily on such evidence as curability by mitomycin C and, in some instances, conjugal transferability. More recently, this has been confirmed by the physical demonstration of plasmid DNA, its use in transformation, restriction fragment analyses, and contour length measurements of double-stranded plasmid DNA isolated by cesium chloride-ethidium bromide density gradient centrifugation (43, 46, 97, 147). The currently postulated clustered chromosomal location for dissimilatory genes in *P. putida*, including those for mandelate utilization (26, 184), based on transductional linkage data with phage pf16h2 might be explained by a plasmid location for these genes. The estimated genetic length of 9% for the dissimilatory genes of the *P. putida* chromosome, using the Wu formula (189) for linkage analyses and assuming the chromosome to be 2.4×10^9 daltons (184), projects these genes to be on a chromosome fragment of 216×10^6 daltons. *Pseudomonas* plasmids of this size or even larger have been

reported for the widely prevalent IncP-2 plasmids, including the CAM and OCT plasmid or *P. putida* (47, 60). The plasmid location for these genes would be in keeping with current views on the advantages of genetic transmissibility (110) via plasmids, especially since it has now been demonstrated that the toluene-degrading genes of a TOL plasmid are transposable (92). There is a pressing need for a more-extensive chromosomal map of *P. putida* and other fluorescent pseudomonads so that the plasmid or chromosomal location of particular dissimilatory functions can be accurately determined.

A study of gene distribution in *P. acidovorans* has recently been initiated for the first time. Heath and colleagues have developed a transductional system using the temperate, generalized transducing phage ϕ12 to study the genetics of histidine utilization (*hut*) and tryptophan biosynthesis (*trp*) (13, 135, 148). A transductional cluster of four *hut* structural genes and a postulated repressor gene *hutC* which is unlinked to the structural genes have been identified in *P. acidovorans*. Physiological and enzyme experiments indicate that the structural genes do not form an operon. The arrangement of the structural gene cluster is analogous to that in *P. putida* (111). Mutations in a regulatory locus lead to enzyme constitutivity. This locus was found to be in the same cluster in *P. putida*, but whether this is an operator site or repressor gene has not been determined. Limited mapping data for *P. aeruginosa* have shown two unlinked regions for genetic expression of histidine utilization, with the loci *hutU* and *hutC* being analogous to those in *P. putida* (Fig. 2; Table 1) (37).

Comparisons are now possible with respect to the *trp* genes in the three species *P. aeruginosa*, *P. putida*, and *P. acidovorans*. Unlike enteric bacteria, in which the *trp* genes form a single operon, there are three unlinked groups of genes in *P. aeruginosa* and *P. putida* (36). In *P. aeruginosa*, *trpA,B* maps at 27 min, *trpC,D,E* maps at 33 min, and *trpF* maps at about 60 min (Fig. 2; Table 1). In *P. putida*, the arrangement of *trp* genes into three clusters is the same as in *P. aeruginosa* (Fig. 1) (26, 137). In *P. acidovorans*, there are only two clusters, *trpF,B,A* and *trpG,D,C* (13, 135), with *trpE* being weakly linked to *trpG,D,C*, the level of recombinants recovered between *trpE* and *trpD* or *trpC* suggesting an interval of a number of genes. The *trpF,B,A* cluster in *P. acidovorans* is linked to a *leu* gene and, as seen in Fig. 2 and 3, there is linkage between *trpF* and a *leu* locus in *P. aeruginosa* and *P. putida* (Fig. 1).

The arrangement of genes controlling tryptophan biosynthesis has now been determined in a number of species, enabling speculation on how this pathway has evolved (36, 135). That variations in arrangement between different species of *Pseudomonas* do occur for this pathway augurs well for the role that gene arrangement studies will play in the understanding of the regulation of gene expression and the evolution of species in this genus.

Amidase system. Aliphatic amidases are a unique group of enzymes elaborated by many strains of *P. aeruginosa* and some strains of *P. putida*, *P. acidovorans*, and *P. cepacia*. The biochemistry with respect to substrate and inducer specificity and the genetics of this enzyme system have been extensively studied by Clarke and her colleagues (see 34 for a review). This system continues to be perhaps the only one in *Pseudomonas* (and specifically in *P. aeruginosa*) in which a combination of genetic fine-structure analysis, studies of regulation of gene expression, and studies of the experimental evolution of novel enzyme variants capable of utilizing novel substrates are being combined to elucidate an enzyme system which is likely to have general significance for *Pseudomonas* and other microorganisms (4, 5, 45). Phage F116 has played an important role in this analysis, since it was early established in transductional crosses using this phage in *P. aeruginosa* strain PAC (previously 8602) that the amidase system is composed of two very closely linked (80 to 100%) cotransducible loci: *amiE*, specifying the structural gene for the enzyme; and *amiR*, the regulator gene (8). More recently, the same phage has been used in three-point transductional crosses to determine gene order between mutations affecting *amiE* and *amiR* (4). These fine-structure genetic studies involving 26 *amiE* mutants have shown that 10 of the mutations map within a very short region of the gene, and it has been postulated that these might be involved in the amide binding capacity of the enzyme. Studies of the amidase system have been extended to *P. aeruginosa* PAO, and a chromosomal location very late in the FP2 transfer sequence has been identified in interrupted-mating conjugal crosses (37) (Table 1; Fig. 2). From a number of lines of evidence, including the behavior of temperature-sensitive mutations affecting *amiR*, it has been concluded that the regulation of *amiE* expression is solely under positive control (45), this being the first example of classical positive control in *Pseudomonas*. These studies in gene regulation need to be confirmed by tests with partial diploids. The isolation of promoter mutants and their chromosomal mapping (164) and the isolation of operator mutants and much more detailed fine-structure mapping of the *ami* genes should considerably enhance the understanding of gene expression in *Pseudomonas*.

Suppressors. Suppressor mutations have played an important role in the elucidation of how genetic information is expressed, and they have provided a highly useful tool for microbial geneticists. Indeed, suppressor mutations, specific for amber, ochre, opal, frameshift, and missense mutations, have been extensively mapped in *E. coli* K-12 and in *S. typhimurium* LT2 (1, 157).

Until recently, such mutations were unknown in *P. aeruginosa*. However, in the last 2 years, a number of independently isolated suppressor mutations have been discovered in *P. aeruginosa* PAT and PAO. In the course of characterizing a temperature-sensitive mutant of a multiple auxotroph of PAT, Watson and Holloway (180) discovered that its presence led to the simultaneous nonrequirement for growth of lysine and adenine. Appropriate transductional crosses indicated that a temperature-sensitive suppressor mutation unlinked to either auxotrophic marker was responsible, and reversion tests indicated that the suppressivity and temperature sensitivity were allelic. The suppressor, designated *sup-1*, was shown to map within cotransductional distance of a cluster of *thr* loci in strain PAT. The isolation of this suppressor facilitated the isolation of suppressible mutations (*sus*) affecting the lytic activity of the virulent phage E79, and *sup-1* was shown to be highly efficient in suppressing the E79 *sus* mutants. Similar suppressors were sought by coreversion of the *lys* and *pur* genes, and these were readily obtained. To determine the genetic basis of *sup-1*, a suppressible mutant of the wide-host-range R plasmid R18 (28) was constructed, and the plasmid was transferred to *E. coli* K-12, for which many well-characterized nonsense suppressors are known (1). However, *supD*, *supC*, or *supF* of *E. coli* was unable to suppress the R18 *sus* mutant, suggesting that *sup-1* of *P. aeruginosa* PAT was not equivalent to the amber or ochre suppressors of *E. coli* K-12. It was tentatively concluded that, due to the broad specificity of suppression by *sup-1*, it was some form of informational suppressor, but more work is required to determine its characteristics.

Other informational suppressors, termed *supA*, *supB*, and *supC*, have been isolated in *P. aeruginosa* PAO by a different procedure (132). A promiscuous plasmid, RP1 was used to first select mutations in the carbenicillin and tetracycline resistance genes of RP1 in *E. coli* K-12 which were simultaneously suppressible by a known *E. coli* amber suppressor (*supD*), and then the plasmid so produced (called pLM2) was transferred to *P. aeruginosa* PAO. From mutagenized stocks of *P. aeruginosa*(pLM2), it was possible to isolate bacterial mutants able to suppress simultaneously both antibiotic markers, and some of these were also able to suppress amber-suppressible mutants of the donor-specific phage PRD1, which had previously been isolated from and identified in *E. coli*. Although neither the genetic basis of the PAO *supB* mutation nor its genetic location was determined, it is fair to assume that it is also an informational suppressor. The isolation of pLM2 should facilitate the isolation of amber-suppressible mutants of chromosomal genes in *P. aeruginosa*, but, as this plasmid retains the wide host properties of RP1, similar mutants can now be isolated in other *Pseudomonas* species.

Kageyama and his colleagues (101a) have also isolated a series of nonsense suppressors in *P. aeruginosa* PAO. With the availability of the in vitro-engineered RP4-*trp* (the *E. coli* K-12 *trp* operon ligated to RP4; 140) they isolated an amber (Am) mutation in one of the *E. coli trp* genes to obtain RP4-*trp*(Am). In *E. coli*, a series of *sus* mutants of the plasmid donor-specific phages PRD1 and PRR1 were isolated which were used in the characterization of nonsense suppressors in *P. aeruginosa* PAO. To isolate such *Pseudomonas* suppressors, they transferred RP4-*trp*(Am) to strain PAO from *E. coli*, the resultant strain, *P. aeruginosa* RP4-*trp*(Am), was screened for suppressibility of the *trp* amber mutation, and pseudo-wild-type prototrophs were then screened for suppression of PRD1 *sus* mutants. Three independent nonsense suppressor (Su^+) mutants of PAO were isolated and characterized with respect to their suppressibility of PRD1 *sus* mutants that had been cataloged as amber or ochre mutants in *E. coli*. The PAO Su^+ strains were found to be amber suppressors, and their pattern of suppression and the behavior of PAO Su^- strains indicated that the phenomenon of informational suppression may be subtly different between *E. coli* K-12 and *P. aeruginosa* PAO. For example, PRD1 *sus* mutants that were typical amber mutants in *E. coli* were, nevertheless, quite capable of forming plaques efficiently on various PAO strains which were apparently Su^-. Conversely, PRR1 *sus* mutants which were efficiently suppressed by *E. coli supD*, *supE*, or *supF* were nonsuppressible by Su^+ strains of PAO. A similar result was also obtained by Mindich et al. (132). Further data will be needed to clarify the relationship between the mechanisms of suppression in *E. coli* and *P. aeruginosa*.

The availability of information suppressors in *P. aeruginosa* PAO has opened the possibility of studying phage and plasmid genetics. Kageyama et al. (101a) have used the *P. aeruginosa* Su^+ strains to isolate *sus* mutants of phages E79 and D3, and the mutants have been shown to

belong to different complementation groups. Carrigan et al. (19) have utilized *sup-1* (180), *supB* (132), and *sup-2* (19; probably identical to *supB*) to characterize transfer-deficient (Tra^-) mutants of the narrow-host-range R plasmid R91-5 of *P. aeruginosa* (29). Using *supB*, Lehrer and Krishnapillai (unpublished data) have isolated and characterized Tra^- *sus* mutants of the wide-host-range IncP-1 plasmid R18. In most instances, the efficiency of suppression was low, although highly suppressible mutants of R18 have been isolated.

PLASMID-CHROMOSOME INTERACTIONS

There are well-documented cases of the integration of plasmid DNA into the bacterial chromosome, notably the plasmid F and bacteriophage lambda in *E. coli*. Other prophages in various bacteria are known to integrate (see below), but the evidence for other plasmid integration is much less extensive. However, according to current studies of transposon behavior (12), it is likely that fragments of plasmids, and perhaps more rarely whole plasmids, do have the property of being integrated into the chromosome and causing permanent alterations to the bacterial phenotype. Such a mechanism has been suggested for the role of degradative plasmids in contributing to genetic variation and evolution in *Pseudomonas*. Certainly the chromosomal integration of prophages and aeruginocinogenicity determinants has been demonstrated, and there are various examples of chromosome-plasmid interactions. The nature of such interactions involved in Cma has been reviewed elsewhere (71).

Location of Prophages in Strain PAO

Prophages may integrate into bacterial chromosomes or exist extrachromosomally as plasmids. The integration of lambda into the *E. coli* chromosome has been intensively studied (52), and precise chromosomal locations in *E. coli* for several prophages are known (1); in *S. typhimurium*, chromosomal locations for eight prophages are known (157). Although some prophages, such as lambda in *E. coli* and P22 in *S. typhimurium*, have single chromosomal integration sites, others insert at more than a single site, as for example the prophage of phage P2, which has 10 integration sites although 1 site predominates with respect to the frequency of integration (14). Chromosomal locations for prophages of other bacteria have been much less extensively studied, but they have been reported for *B. subtilis* (192) and in *Streptomyces coelicolor* (82). Extrachromosomal locations have been shown for prophages P1, P7, and N15 of *E. coli* (63, 86, 153, 190) by means of a variety of physical methods. Recently the prophage of phage Rφ6P of *Rhodopseudomonas sphaeroides* has also been shown to exist as a plasmid (177).

Chromosomal locations have been shown for prophages in *P. aeruginosa* PAO (17, 18, 106; K. E. Carey, Ph.D. thesis, Monash University, Clayton, Australia, 1974). Both plate matings and interrupted-mating experiments with FP2 donors have placed the H90 prophage between *car-9* (9 min) and *argA* (18 min) (Fig. 2; Table 1). Cotransduction of the prophage with any other marker was not found. In crosses between donors lysogenic for H90 and nonlysogenic recipients, zygotic induction (89) was found to occur after transfer of the prophage, which affected recovery of markers distal to the prophage site. However, this was not accompanied by any increase in plaque-forming particles, and together with other data (Carey, Ph.D. thesis) this indicates that phage H90 is partially defective. Electron microscope examination of phage lysates shows that the proportion of apparently fully assembled phage particles to unassembled phage components is 1:90. Further work has shown that phages very closely related to H90 are indeed very common among temperate phages isolated from wild-type hospital strains of *P. aeruginosa*, and all are chromosomally located in the same region as H90 (Carey, Ph.D. thesis).

Two other temperate phages have also been shown to have a chromosomal location for their prophages (18; Carey, Ph.D. thesis). The ultraviolet-inducible phages J51 and J84 are related by the criteria of homoimmunity and serological cross-reactivity, but J51 is analogous to lambda because of its zygotic induction behavior. There is a reduction in the recovery of zygotes inheriting markers distal to the prophage location site, and also there is a concomitant increase in infectious centers (18). This has enabled the use of infectious centers as an assay for the entry of the prophage site in interrupted matings between lysogenic donors and nonlysogenic recipients. The chromosomal site for prophage J51 has been located close to 50 min on the PAO chromosome (18). The prophage of J84 also appears to map in this region by analysis of coinheritance of prophage J84 with other bacterial markers in plate-mating crosses; however, unlike J51, J84 is zygotically noninducible and therefore a more-precise location was not determinable (Carey, Ph.D. thesis). However, the lack of infectious center increase in interrupted matings between J84 lysogenic donors and nonlysogenic recipients is analogous to the behavior of the *E. coli* prophage 186, which is also ultraviolet inducible but zygotically noninducible (186).

Genetic mapping experiments (Carey, Ph.D. thesis) have so far failed to provide evidence for the chromosomal locations of the better-characterized temperate phages B3, D3, G101, and F116L of *P. aeruginosa* (76). Miller et al. (130), using F116, which was isolated from the same lysogenic strain of *P. aeruginosa* as F116L, presented evidence for the mobilization of the F116 prophage by the FP2 plasmid in noninterrupted matings, which has been interpreted as resulting from a plasmid-like or extrachromosomal location for the F116 prophage. This conclusion was also supported by the appearance of satellite DNA in CsCl gradients of tritium-labeled DNA from lysogens (130).

Location of Aeruginocinogenicity Determinants

Like lysogeny, aeruginocinogenicity (pyocinogenicity) is extremely common in *P. aeruginosa*. Two types of aeruginocins can be distinguished: R type, which have a shape similar to a bacteriophage tail, with a single protein subunit of molecular weight 10^7; and the S type, which are proteins of molecular weight ca. 75,000 (76).

Kageyama and his colleagues (98–101, 162) have mapped the chromosomal location in *P. aeruginosa* PAO of the genetic determinants coding for R bacteriocins. A variety of R-type aeruginocins are found in *P. aeruginosa* strains from different geographical origins, but they appear to be remarkably similar with respect to morphology, mode of action, and immunological properties. Kageyama and his co-workers have identified R-type aeruginocins designated R1, R2, and R3 in *P. aeruginosa* strains P15 (isolated in Japan), PAO (Australia,) and PAT (South Africa), respectively. The genetic determinants for R1 have been shown by FP2-mediated conjugation and F116-mediated transduction to be located at about 32 to 34 min on the PAO chromosome (98, 99). By similar genetic crosses, the determinants for R3 were transferred into PAO from PAT. Recombinational analysis has shown that the R2 and R3 determinants are very likely allelic (101). Similarly, R1 is likely to be allelic to R2 (100). These R-type aeruginocin genetic determinants may thus have a common ancestry, and one possibility is that a normal prophage was ancestrally located at this chromosomal site and subsequently became defective. Such a postulated relationship between the genetic determinants of R-type aeruginocins and normal prophages is strengthened by the serological similarity between R-type aeruginocins and normal temperate phages of *P. aeruginosa* (81). The single chromosome site for the location of these determinants has its counterpart in the prophage of the defective phage PBSX of *Bacillus subtilis*, which also has a singular site (175). The location of the S-type aeruginocinogenicity determinant has not been determined. They have not been transmitted in conjugation, nor have attempts at curing been successful.

In summary, it appears well established that prophages and bacteriocinogenicity determinants have specific locations on the chromosome of *P. aeruginosa* PAO. In particular three regions, 7 to 18 min, 32 to 34 min, and 50 min, show a preference for such locations. The demonstration of chromosomal locations of prophages in *P. aeruginosa* opens the way to the exploitations of this knowledge for the development of specialized transduction systems as, for example, with ϕ80 in *E. coli* K-12, where the construction of transductional heterogenotes facilitated the study of the regulation of the tryptophan operon (123).

Other Plasmid-Chromosome Interactions

It is known that IncP-1 plasmids are stably maintained in *P. aeruginosa* PAO but are unstable in strain PAT, suggesting a role for the bacterial genome in maintenance of plasmids. This instability manifests itself as the progressive loss of plasmid markers at a frequency of about 1% per cell generation, resulting eventually in plasmid-free cells (73), a phenomenon which has been termed structural instability (71). Both instability and Cma are abolished in recombination-deficient hosts but reappear in Rec^+ revertants, selected on the basis of resistance to mitomycin C (A. J. Godfrey and A. F. Morgan, manuscript in preparation). The instability is due to interaction with pVS1, a resident nonconjugative PAT plasmid (168), and is strain independent since IncP-1 plasmids are unstable in PAO hosts carrying pVS1. However, in PAO, instability is not accompanied by Cma, and hence there must also be chromosomal differences between PAO and PAT in the IncP-1 origin region (Godfrey and Morgan, in preparation).

The requirement of a functional recombination system for instability to occur theoretically gives a very strong selection method for isolating Rec^- mutants or any other chromosomal mutants affecting the process. However, no such mutants have been found, and so it is possible that it is a lethal event for the cell if such mutants are generated while the IncP-1 plasmid is present. Reconstruction experiments, in which Rec^+ and Rec^- PAT strains carrying the IncP-1 plasmid R68 were mixed at a ratio of 10^6:1,

yielded a high proportion of Rec$^-$ cells after only a few cycles of alternate selection and relaxation (Godfrey and Morgan, in preparation).

Chang and Holloway (30) isolated a chromosomal mutation, *risA*, which renders the host temperature sensitive for the maintenance of some but not all IncP-2 plasmids, although plasmids of other incompatibility groups are not affected. The kinetics of plasmid loss suggested that plasmid-containing cells are unable to grow at the nonpermissive temperature but are overgrown by plasmid-deficient cells. Nakazawa (141) has shown a similar effect with the TOL plasmid in *P. aeruginosa* PAO.

GENETIC BASIS OF RESISTANCE TO ANTIBIOTICS AND OTHER TOXIC AGENTS

P. aeruginosa has the reputation for being an unusually resistant organism in terms of its response to a variety of antibiotic and chemotherapeutic agents. In recent years, the emphasis on the genetic basis of this resistance has been in terms of plasmids, and there are a number of extensive accounts of resistance plasmids in *P. aeruginosa* (91, 93, 167). A discussion of the resistance of *Pseudomonas* in terms of antibiotic inactivation, the role of the cell envelope in resistance, and the ultrastructure of *P. aeruginosa* as related to resistance (9) indicates that there are a variety of mechanisms by which *P. aeruginosa* can display resistance to antibiotics other than by plasmid-coded functions, but it is surprising that so little is known about the genetic basis of this type of resistance.

As described earlier, Watson and Holloway (182) have demonstrated a cluster of genes determining antibiotic resistance in *P. aeruginosa* PAT, which includes rifampin, streptomycin, and spectinomycin, as well as hypersensitivity to fusidic acid (Fig. 3). Other locations for genes affecting aminoglycoside resistance have been identified in *P. aeruginosa* PAO. L. E. Bryan (unpublished data) has identified a locus, *aglA*, in strain PAO which shows a higher resistance to a variety of aminoglycosides, and this is cotransducible with *ilvB/C* at about 8 min. C. E. Crowther and B. W. Holloway (unpublished data) have isolated spontaneous mutants resistant to kanamycin which also show resistance to other aminoglycosides, and this marker (designated *aglD*) has been shown to be cotransducible with *argC* (35 min) and, hence, is in the same region as the other ribosomally involved resistance mutations mentioned above. A third type of aminoglycoside resistance has been demonstrated in a strain of *P. aeruginosa* carrying the ECM plasmid R68.45. For this strain, mutants resistant to high-level streptomycin occurred spontaneously at 50 to 100 times the normal frequency. When these were characterized, it was found that they were highly resistant to all aminoglycosides tested, and a locus *aglE* responsible for this form of resistance has been mapped in the vicinity of *argF* (75; Crowther and Holloway, unpublished data). It is of interest that most *aglE* mutants derived from R68.45-containing strains have also lost Cma. In addition, it has been shown that curing of the R68.45 plasmid from such strains results in loss of the mutator ability for this mutation. It is tempting to speculate that the region of the R68.45 plasmid which is thought to be responsible for Cma, namely, the 1,800-base pair insertion near the locus for kanamycin resistance (van Montagu and Schell, personal communication; Reiss, Burkhardt, and Pühler, personal communication), is acting as a mutator element by excising from the plasmid and becoming inserted into the chromosome.

The nature of aminoglycoside resistance in *P. aeruginosa* in particular and gram-negative bacteria in general has been considered by Bryan and van den Elzen (10), and, through the study of aminoglycoside uptake in a variety of mutants, a model has been formulated for aminoglycoside entry in bacteria which involves a low-affinity membranous complex involved in membrane energization. This includes respiratory quinones, these acting to bind and transport aminoglycosides across the cell membrane. Evidence to support this theory comes from the reaction to antibiotics of a variety of *E. coli* mutants which are altered in ubiquinone synthesis, such as *ubiD* mutants, which synthesize about 20% of the normal quantity of ubiquinone and exhibit increased resistance to all aminoglycosides and cannot accumulate gentamicin or streptomycin. Additional support comes from studies of mutants, both of *E. coli* and *P. aeruginosa*, in which there is alteration in response to bacteriocins and, hence, very likely in the nature of the cytoplasmic membrane (68). Mills and Holloway (131) have described the isolation of an aeruginocin-tolerant mutant of *P. aeruginosa* PAO which shows hypersensitivity to all aminoglycosides tested and which has not changed its susceptibility to a wide range of other toxic agents, including antibiotics and surfactants. The locus responsible for this change, *tolA*, has been mapped at about 10 min from the FP2 origin and is closely linked to *car-9*. Evidence involving the *tolA* locus in this specific hypersensitivity was obtained by selecting for revertants of the hypersensitive phenotype when revertants to *tol*$^+$ were found (131). It has not yet

been possible to correlate structural changes in such aeruginocin-tolerant mutants with changes in energization, but the properties of this mutant clearly indicate an approach for the experimental testing of Bryan and van den Elzen's model.

GENETIC ANALYSIS OF CELL STRUCTURE

Although microbiologists agree that *Pseudomonas* shows a remarkable resistance to a wide variety of toxic agents, as yet there is no consensus as to how this is achieved. Analysis of the *Pseudomonas* cell envelope has so far not revealed any special features to which this resistance could be ascribed (for a review see 9). However, only relatively recently have the isolation and genetic analysis of cell envelope mutants been used as an approach to this problem, which is encouraging in view of the success that such an approach has achieved in other gram-negative bacteria, notably *Salmonella* (173).

Lipopolysaccharide-defective mutants of *P. aeruginosa* PAC have been isolated by selection for resistance to aeruginocin and, by analysis of different mutants, evidence has been obtained showing the existence of a core polysaccharide with different amounts and kinds of side chains (104). A novel feature of these experiments was the use of bacteriocins to select spontaneous lipopolysaccharide-defective mutants, an approach not commonly used with enteric bacteria. Kropinski and his co-workers (108) have isolated lipopolysaccharide-specific phages PB1, φPRS-37, and φPRS-43 (94, 107). A comparison of the lipopolysaccharide from wild-type strain PAO and a rough mutant resistant to the virulent phage E79 showed that the mutant lacked side-chain material and was defective in its inner core region (95). This is interesting in view of the demonstration that E79 is a generalized transducing phage. Two *ese* (E79 resistance) loci have been mapped (Fig. 2; Table 1). Markers involved in somatic antigen formation have also been mapped on the *P. aeruginosa* PAO chromosome (122).

A genetic analysis of flagellum formation has been initiated (T. Iino and T. Suzuki, personal communication). Six mutation sites, *flaA* through *flaE* and *hag*, have been identified, with *hag* being the structural gene for flagellin. The six loci are all closely linked, as shown by F116 transduction. An additional mutation, responsible for multi-flagellate mutants, also maps in the *fla* gene cluster.

MICROBIAL GENETICS IN MEDICAL MICROBIOLOGY

We have already discussed the role that formal genetic analysis may play in the understanding of antibiotic resistance, both chromosomally and plasmid determined, in *P. aeruginosa*. However, there are a number of other medical problems involving *P. aeruginosa* for which a genetic approach is yielding profitable results. One of these concerns mucoid strains of *P. aeruginosa*, these being strains which excrete a polysaccharide such that colonies become viscous and large and the bacterial cells acquire a capsule of extruded polysaccharide. It has been found that over 90% of patients suffering from cystic fibrosis die of progressive pulmonary involvement complicated by infection with *P. aeruginosa*. Doggett et al. (40) observed that mucoid isolates of *P. aeruginosa* could be isolated from 70% of cystic fibrotic patients, an observation later extensively confirmed by other workers (39, 65, 154). Certain features of these mucoid variants are of interest. Whereas such mucoid strains generally emerge and persist in vivo, particularly in cystic fibrotic patients, they revert to the nonmucoid form in vitro (53, 54, 118). Govan and Fyfe (55) have described techniques for isolating mucoid variants in vitro by selective techniques involving mutants resistant to carbenicillin, flucloxacillin, and tobramycin, the technique involving careful selection of the level of antibiotics to be used. The frequency of isolation of such mucoid variants was approximately 10^{-7} and could be increased 40-fold by mutagenesis of nonmucoid strains with ethyl methane sulfonate. Similar variants were obtained after selection with the virulent phage E79 (53) and also by selection for colonies surviving treatment with aeruginocins (H. Rossiter and B. W. Holloway, unpublished data). Characterization of the antibiotic- and E79-resistant mucoid variants showed them to be indistinguishable from mucoid *P. aeruginosa* strains isolated from patients with cystic fibrosis (55). Markowitz et al. (117) have presented evidence that exopolysaccharide production does not appear to be plasmid linked and have begun the construction of strains to be used for exploring the genetic basis of the mucoid phenotype. Govan (53) and Fyfe and Govan (51), in a similar approach with crosses involving both FP2 and R68.45 in strain PAO, have indicated that chromosomal locations for mucoid determinants can be assigned on the basis of such crosses and, hence, exopolysaccharide production by *P. aeruginosa* takes place after a chromosomal mutation. For such genetic studies, it is essential that the mucoid variants remain stable in culture; Govan (53) has indicated that stability is improved when the mucoid variant is grown in the presence of lecithin or sodium deoxycholate. By means of fluctuation tests and growth rate

experiments, Govan et al. (56) found that the spontaneous reversion of the mucoid form to the wild-type form behaved, in a fluctuation test, as if a spontaneous mutation was occurring, and by studying the growth rates of mucoid and nonmucoid forms, it was found that there was a very real growth rate advantage for the nonmucoid revertants, particularly in cultures in which aeration was limiting. Under conditions in which aeration is not limited, as in shaken cultures, there is little difference in the growth rate between the mucoid and nonmucoid forms.

Genetic analysis is also being applied to the study of virulence of *P. aeruginosa*. It is known that this organism produces a range of extracellular compounds, and efforts are being made to relate individual compounds to virulence. The most likely virulence factor is a trypsin-sensitive, heat-labile, protein exotoxin (113). Iglewski and Kabat (84) have shown that this toxin, toxin A, causes a block in an elongation step of polypeptide assembly and in this respect acts like diphtheria toxin fragment A. Subsequently, Iglewski et al. (85) identified another enzyme secreted by *P. aeruginosa* which is an adenosine diphosphate ribosyltransferase that is distinct from exotoxin A. The location of the structural genes of these products is not known, but 80% of all isolates of *P. aeruginosa* tested produce exotoxin A, so that the structural gene for this enzyme is either on the chromosome or on a plasmid which is common to many natural strains of *P. aeruginosa*. The genetic study of these exoenzymes should be particularly interesting and perhaps one in which an ECM plasmid like R68.45 could be effectively used.

SUMMARY AND PROSPECT

The ability to recognize and manipulate the chromosomal genes of pseudomonads is attractive for both genetic and microbiological purposes. The diversity of these organisms has provided an extensive forum for conjecture by those interested in microbial evolution. Work by Ornston and his colleagues on the evolution of the β-ketoadipate pathway provides the best evidence at the amino acid sequence level as to how metabolic pathways are acquired. Yeh et al. (191) have shown that the muconolactone isomerases of *Pseudomonas* and *Acinetobacter* are evolutionarily homologous and evidently share a common ancestral gene. By the immunological comparison of γ-carboxymuconolactose decarboxylases from various *Pseudomonas* species, Patel and Ornston (149) concluded that interspecific transfer of the structural gene for this enzyme does not commonly occur.

Genetically, the pseudomonads can be considered at the present time to consist of two types. One is *P. aeruginosa*, for which plasmids with and without Cma are common and which is almost invariably lysogenic for bacteriophages, many of which are transducing. By contrast, *P. putida* and similar fluorescent pseudomonads are only rarely lysogenic, transducing phages are rare, and plasmids do occur but are not very common. Where found, these plasmids have a selective value predominantly in terms of catabolic activity and only infrequently show Cma. Finding and developing practical systems of genetic analysis for *P. putida* and other fluorescent pseudomonads has not been easy. The discovery that IncP-1 plasmids possess the machinery to transfer genetic material raises the question as to their role, if any, in the evolution of different species of this genus. Perhaps the IncP-1 plasmids are a relatively recent experiment in evolution, or they have been isolated from the fluorescent pseudomonads by geographical or other spatial barriers. Alternatively, the selective pressure of antibiotics has been needed to overcome some natural disadvantage that IncP-1 plasmids possess. The use of IncP-1 plasmids with Cma in interspecific crosses may provide essential genetic evidence which will complement the important evolutionary findings by Ornston referred to above. Hopefully, the use of such plasmids may provide additional ways of determining genetic similarity and dissimilarity of particular taxonomic groups.

An important experimental avenue which must be explored is the integration of plasmid material into the bacterial chromosome, particularly with respect to genes determining substrate utilization. It is difficult to speculate on the evolutionary significance of this aspect of the pseudomonad genome without knowing whether such DNA can only be transferred from plasmid to plasmid (as has been shown with TOL and SAL [22, 92]) or whether such genetic information can be added stably and without loss of essential function to the bacterial chromosome. Knowledge of insertion sequences and transposons of *Pseudomonas* and related genera will be essential for the complete understanding of how genetic material can move between the various species of the genus.

For what may be purely practical reasons, the properties of IncP-1 plasmids (possession of Cma, ability to generate R′ plasmids, and extensive bacterial host range) may enable the realization of the industrial potential for *Pseudomonas*, which has been widely acclaimed but never quite realized (72). Already, one multiplasmid strain of *P. putida* has been patented for purposes of pollution control (49). *Pseudomonas* species are being tested for their role in the manufacture of single-cell protein, using

methanol or ethanol as a growth substrate (112). A new area for potential development is the construction of *Pseudomonas* recombinants for the production of desired byproducts which are too expensive to obtain by standard industrial chemical processes. The metabolic versatility of the genus and the ability to grow on inexpensive, readily available substrates, combined with in vitro and in vivo genetic engineering procedures, could be the recipe for a major industrial development in the early twenty-first century, when traditional sources of raw material may have declined.

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LITERATURE CITED

1. **Bachmann, B. J., K. B. Low, and A. L. Taylor.** 1976. Recalibrated linkage map of *Escherichia coli* K-12. Bacteriol. Rev. **40**:116–167.
2. **Barksdale, L., and S. B. Arden.** 1974. Persisting bacteriophage infections, lysogeny, and phage conversions. Annu. Rev. Microbiol. **28**: 265–299.
3. **Benson, S., and J. Shapiro.** 1978. TOL is a broad-host-range plasmid. J. Bacteriol. **135**: 278–280.
4. **Betz, J. L., J. E. Brown, P. H. Clarke, and M. Day.** 1974. Genetic analysis of amidase mutants of *Pseudomonas aeruginosa*. Genet. Res. **23**:335–359.
5. **Betz, J. L., P. R. Brown, M. J. Smyth, and P. H. Clarke.** 1974. Evolution in action. Nature (London) **247**:261–264.
6. **Blevins, W. T., T. W. Feary, and P. V. Phibbs, Jr.** 1975. 6-Phosphogluconate dehydratase deficiency in pleiotropic carbohydrate-negative mutant strains of *Pseudomonas aeruginosa*. J. Bacteriol. **121**:942–949.
7. **Boucher, C., and L. Sequeira.** 1978. Evidence for the cotransfer of genetic markers in *Pseudomonas solanacearum*. Can. J. Microbiol. **24**: 69–72.
8. **Brammar, W. J., P. H. Clarke, and A. J. Skinner.** 1967. Biochemical and genetic studies with regulator mutants of the *Pseudomonas aeruginosa* 8602 amidase system. J. Gen. Microbiol. **47**:87–102.
9. **Brown, M. R. W. (ed.).** 1975. Resistance of *Pseudomonas aeruginosa*. J. Wiley and Sons, London.
10. **Bryan, L. E., and H. M. van den Elzen.** 1977. Effects of membrane-energy mutations and cations on streptomycin and gentamicin accumulation by bacteria: a model for entry of streptomycin and gentamicin in susceptible and resistant bacteria. Antimicrob. Agents Chemother. **12**:163–177.
11. **Bryan, L. E., H. Semaka, and H. van den Elzen.** 1973. Characteristics of R931 and other *Pseudomonas aeruginosa* R factors. Antimicrob. Agents Chemother. **3**:625–637.
12. **Bukhari, A. I., J. A. Shapiro, and S. L. Adhya (ed.).** 1977. DNA insertion elements, plasmids, and episomes. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

12a. **Burkhardt, H. J., G. Riess, and A. Pühler.** 1978. Molecular relationship between R plasmids RP1, RP4, RP8, RK2, R68 and R68.45 revealed by electron microscopical techniques. Hoppe-Seyler's Z. Physiol. Chem. **359**:1068.

13. **Buvinger, W. E., and H. E. Heath.** 1979. Organization of the *trp* genes in *Pseudomonas acidovorans*. J. Bacteriol. (submitted).
14. **Calendar, R., E. W. Six, and F. Kahn.** 1977. Temperate coliphage P2 as an insertion element, p. 395–402. *In* A. I. Bukhari, J. A. Shapiro, and S. L. Adhya (ed.), DNA insertion elements, plasmids, and episomes. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
15. **Calhoun, D. H., D. L. Pierson, and R. A. Jensen.** 1973. The regulation of tryptophan biosynthesis in *Pseudomonas aeruginosa*. Mol. Gen. Genet. **121**:117–132.
16. **Campos, J. M., J. Geisselsoder, and D. R. Zusman.** 1978. Isolation of bacteriophage MX4, a generalised transducing phage for *Myxococcus xanthus*. J. Mol. Biol. **119**:167–178.
17. **Carey, K. E., and V. Krishnapillai.** 1974. Location of prophage H90 on the chromosome of *Pseudomonas aeruginosa* strain PAO. Genet. Res. **23**:155–169.
18. **Carey, K. E., and V. Krishnapillai.** 1975. Chromosomal location of prophage J51 in *Pseudomonas aeruginosa* strain PAO. Genet. Res. **25**: 179–187.
19. **Carrigan, J. M., Z. M. Helman, and V. Krishnapillai.** 1978. Transfer-deficient mutants of the narrow-host-range plasmid R91-5 of *Pseudomonas aeruginosa*. J. Bacteriol. **135**:911–919.
20. **Chakrabarty, A. M.** 1976. Plasmids in Pseudomonas. Annu. Rev. Genet. **10**:7–30.
21. **Chakrabarty, A. M., and D. A. Friello.** 1974. Dissociation and interaction of individual components of a degradative plasmid aggregate in *Pseudomonas*. Proc. Natl. Acad. Sci. U.S.A. **71**:3410–3414.
22. **Chakrabarty, A. M., D. A. Friello, and L. H. Bopp.** 1978. Transposition of plasmid DNA segments specifying hydrocarbon degration and their expression in various microorganisms. Proc. Natl. Acad. Sci. U.S.A. **75**:3109–3112.
23. **Chakrabarty, A. M., and I. C. Gunsalus.** 1969. Autonomous replication of a defective transducing phage in *Pseudomonas putida*. Virology **38**:92–104.

24. **Chakrabarty, A. M., and I. C. Gunsalus.** 1969. Defective phage and chromosome mobilization in *Pseudomonas putida*. Proc. Natl. Acad. Sci. U.S.A. **64**:1217-1223.
25. **Chakrabarty, A. M., and I. C. Gunsalus.** 1970. Transduction and genetic homology between *Pseudomonas* species *putida* and *aeruginosa*. J. Bacteriol. **103**:830-832.
26. **Chakrabarty, A. M., C. F. Gunsalus, and I. C. Gunsalus.** 1968. Transduction and the clustering of genes in fluorescent pseudomonads. Proc. Natl. Acad. Sci. U.S.A. **60**:168-175.
27. **Chakrabarty, A. M., J. R. Mylroie, D. A. Friello, and J. G. Vacca.** 1975. Transformation of *Pseudomonas putida* and *Escherichia coli* with plasmid-linked drug resistance factor DNA. Proc. Natl. Acad. Sci. U.S.A. **72**:3647-3651.
28. **Chandler, P. M., and V. Krishnapillai.** 1974. Phenotypic properties of R factors of *Pseudomonas aeruginosa*: R factors readily transferable between *Pseudomonas* and the Enterobacteriaceae. Genet. Res. **23**:239-250.
29. **Chandler, P. M., and V. Krishnapillai.** 1977. Characterization of *Pseudomonas aeruginosa* derepressed R plasmids. J. Bacteriol. **130**:596-603.
30. **Chang, B. J., and B. W. Holloway.** 1977. Bacterial mutation affecting plasmid maintenance in *Pseudomonas aeruginosa*. J. Bacteriol. **130**: 943-945.
31. **Clarke, P. H.** 1978. Experiments in microbial evolution, p. 137-218. *In* L. N. Ornston and J. R. Sokatch (ed.), The bacteria, vol. 6, Bacterial diversity. Academic Press Inc., New York.
32. **Clarke, P. H.** 1979. Regulation of enzyme synthesis in the bacteria: a comparative and evolutionary study. *In* R. Goldberger (ed.), Biological regulation and development, vol 1. Plenum Publishing Corp, New York. (In press.)
33. **Clarke, P. H.** 1979. The utilization of amides by microorganisms. *In* J. W. Payne (ed.), Transport and utilization of amino acids, peptides and proteins by microorganisms. John Wiley & Sons Ltd., Chichester, England. (In press.)
34. **Clarke, P. H., and L. N. Ornston.** 1975. Metabolic pathways and regulation—parts I and II, p. 191-340. *In* P. H. Clarke and M. H. Richmond (ed.), Genetics and biochemistry of *Pseudomonas*. John Wiley & Sons, London.
35. **Cosloy, S. D., and M. Oishi.** 1973. The nature of the transformation process in *Escherichia coli* K12. Mol. Gen. Genet. **124**:1-10.
36. **Crawford, I. P.** 1975. Gene arrangements in the evolution of the tryptophan synthetic pathway. Bacteriol. Rev. **39**:87-120.
37. **Day, M., R. J. Potts, and P. H. Clarke.** 1975. Location of genes for the utilization of acetamide, histidine and proline on the chromosome of *Pseudomonas aeruginosa*. Genet. Res. **25**: 71-78.
38. **Dean, H. F., P. Royle, and A. F. Morgan.** 1979. Detection of FP plasmids in hospital isolates of *Pseudomonas aeruginosa*. J. Bacteriol. **138**: 249-250.
39. **Doggett, R. G.** 1969. Incidence of mucoid *Pseudomonas aeruginosa* from clinical sources. Appl. Microbiol. **18**:936-937.
40. **Doggett, R. G., G. M. Hornsai, R. N. Stillwell, and E. S. Wallis.** 1966. An atypical *Pseudomonas aeruginosa* associated with cystic fibrosis of the pancreas. J. Pediatr. **68**:215-221.
41. **Domaradskÿ, I. V.** 1977. The participation of R plasmids in gene transfer. J. Microbiol. Epidemiol. Immunobiol. (U.S.S.R.) **13**:3-10.
42. **Domaradskÿ, I. V., S. F. Borinova, E. V. Filkova, B. S. Sitkinov, and T. B. Levadnaya.** 1976. Intergenera transmission of chromosomal genes with the aid of the RP factor of *Ps. aeruginosa*. J. Microbiol. Epidemiol. Immunobiol. (U.S.S.R.) **11**:59-64.
43. **Duggleby, C. J., G. A. Bayley, M. J. Worsey, P. A. Williams, and P. Broda.** 1977. Molecular sizes and relationships of TOL plasmids in *Pseudomonas*. J. Bacteriol. **130**:1274-1280.
44. **Fargie, B., and B. W. Holloway.** 1965. Absence of clustering of functionally related genes in *Pseudomonas aeruginosa*. Genet. Res. **6**:284-299.
45. **Farin, F., and P. H. Clarke.** 1978. Positive regulation of amidase synthesis in *Pseudomonas aeruginosa*. J. Bacteriol. **135**:379-392.
46. **Farrell, R., I. C. Gunsalus, I. P. Crawford, J. B. Johnston, and J. Ito.** 1978. Restriction endonuclease sites and aromatic metabolic plasmid structure. Biochem. Biophys. Res. Commun. **81**:411-416.
47. **Fennewald, M., W. Prevatt, R. Meyer, and J. Shapiro.** 1978. Isolation of IncP-2 plasmid DNA from *Pseudomonas aeruginosa*. Plasmid **1**:164-173.
48. **Filkova, E. V., N. E. Berezkina, and I. V. Domaradskÿ.** 1977. Transgenosis under the influence of the plasmid RP1: indication of the presence of the "aggregated plasmid" in the intergeneric *Escherichia coli* hybrid. Bull. Exp. Biol. (U.S.S.R.) **9**:360-362.
49. **Friello, D. A., J. A. Mylroie, and A. M. Chakrabarty.** 1975. Use of genetically engineered multiplasmid microorganisms for rapid degradation of fuel hydrocarbons, p. 205-214. *In* J. M. Sharply and A. M. Kaplan (ed.), Proceedings of the Third International Biodegradation Symposium. Applied Science Publishers, London.
50. **Fulbright, D. W., and J. V. Leary.** 1978. Linkage analysis of *Pseudomonas glycinea*. J. Bacteriol. **136**:497-500.
51. **Fyfe, J. A. M., and J. R. W. Govan.** 1978. A genetic approach to the study of mucoid *Pseudomonas aeruginosa*. Proc. Soc. Gen. Microbiol. **5**:54.
52. **Gottesman, M. E., and R. A. Weisberg.** 1971. Prophage insertion and excision, p. 113-138. *In* A. D. Hershey (ed.), The bacteriophage lambda. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
53. **Govan, J. R. W.** 1975. Mucoid strains of *Pseu-*

domonas aeruginosa: the influence of culture medium on the stability of mucus production. J. Med. Microbiol. **8**:513–522.

54. **Govan, J. R. W.** 1976. Antibiotic therapy and cystic fibrosis: increased resistance of mucoid *Pseudomonas aeruginosa* to carbenicillin. J. Antimicrob. Chemother. **2**:215–217.
55. **Govan, J. R. W., and J. A. M. Fyfe.** 1978. Mucoid *Pseudomonas aeruginosa* and cystic fibrosis: resistance of the mucoid form to carbenicillin, flucoxacillin and tobramycin and the isolation of mucoid variants *in vitro*. J. Antimicrob. Chemother. **4**:233–240.
56. **Govan, J. R. W., J. A. M. Fyfe, and C. McMillan.** 1979. The instability of mucoid *Pseudomonas aeruginosa*-fluctuation test and improved stability of the mucoid form in shaker culture. J. Gen. Microbiol. **110**:229–232.
57. **Haas, D., and B. W. Holloway.** 1976. R factor variants with enhanced six factor activity in *Pseudomonas aeruginosa*. Mol. Gen. Genet. **144**:243–251.
58. **Haas, D., and B. W. Holloway.** 1978. Chromosome mobilization by the R plasmid R68.45: a tool in *Pseudomonas* genetics. Mol. Gen. Genet. **158**:229–237.
59. **Haas, D., B. W. Holloway, A. Schomböck, and T. Leisinger.** 1977. The genetic organization of arginine biosynthesis in *Pseudomonas aeruginosa*. Mol. Gen. Genet. **154**:7–22.
60. **Hansen, J. B., and R. H. Olsen.** 1978. IncP-2 group of *Pseudomonas*, a class of uniquely large plasmids. Nature (London) **274**:715–717.
61. **Hedges, R. W., and A. E. Jacob.** 1977. *In vivo* translocation of genes of *Pseudomonas aeruginosa* onto a promiscuously transmissible plasmid. FEMS Microbiol. Lett. **2**:15–19.
62. **Hedges, R. W., A. E. Jacob, and I. P. Crawford.** 1977. Wide ranging plasmid bearing the *Pseudomonas aeruginosa* tryptophan synthase genes. Nature (London) **267**:283–284.
63. **Hedges, R. W., A. E. Jacob, P. T. Barth, and N. J. Grinter.** 1975. Compatibility properties of P1 and ϕ Amp prophages. Mol. Gen. Genet. **141**:263–267.
64. **Heil, A., and W. Zillig.** 1970. Reconstitution of bacterial DNA-dependent RNA polymerase from isolated subunits as a tool for the elucidation of the role of the subunits in transcription. FEBS Lett. **11**:165–168.
65. **Hiøby, N.** 1975. Prevalence of mucoid strains of *Pseudomonas aeruginosa* in bacteriological specimens from patients with cystic fibrosis and patients with other diseases. Acta Pathol. Microbiol. Scand. **83**:549–552.
66. **Holloway, B. W.** 1956. Self fertility in *Pseudomonas aeruginosa*. J. Gen. Microbiol. **15**:221–224.
67. **Holloway, B. W.** 1969. Genetics of *Pseudomonas*. Bacteriol. Rev. **33**:419–443.
68. **Holloway, B. W.** 1971. A genetic approach to the study of the bacterial membrane. Aust. J. Exp. Biol. Med. Sci. **49**:429–434.
69. **Holloway, B. W.** 1975. Genetic organization of *Pseudomonas*, p. 133–161. *In* P. H. Clarke and M. H. Richmond (ed.), Genetics and biochemistry of *Pseudomonas*. John Wiley & Sons, London.
70. **Holloway, B. W.** 1978. Isolation and characterization of an R′ plasmid in *Pseudomonas aeruginosa*. J. Bacteriol. **133**:1078–1082.
71. **Holloway, B. W.** 1979. Plasmids that mobilize bacterial chromosome. Plasmid **2**:1–19.
72. **Holloway, B. W.** 1979. The genetics of pseudomonas. Proceedings of the Fourteenth International Congress of Genetics, Moscow, 1978. (In press.)
73. **Holloway, B. W., C. Crowther, A. Godfrey, D. Haas, V. Krishnapillai, A. F. Morgan, and J. M. Watson.** 1977. Plasmid-bacterial genome interactions in *Pseudomonas aeruginosa*, p. 77–88. *In* S. Mitsuhashi, L. Rosival, and V. Krčméry (ed.), Plasmids: medical and theoretical aspects. Springer-Verlag New York, Inc., New York.
74. **Holloway, B. W., J. B. Egan, and M. Monk.** 1960. Lysogeny in *Pseudomonas aeruginosa*. Aust. J. Exp. Biol. **38**:321–330.
75. **Holloway, B. W., D. Haas, and A. F. Morgan.** 1979. Interactions between R plasmids and the bacterial chromosome. *In* S. Mitsuhashi (ed.), Proceedings of the Second Tokyo Symposium on Microbial Drug Resistance. Japanese Scientific Societies Press, Tokyo. (In press.)
76. **Holloway, B. W., and V. Krishnapillai.** 1975. Bacteriophages and bacteriocins, p. 99–132. *In* P. H. Clarke and M. H. Richmond (ed.), Genetics and biochemistry of *Pseudomonas*. John Wiley & Sons, London.
77. **Holloway, B. W., V. Krishnapillai, and V. Stanisich.** 1971. Pseudomonas genetics. Annu. Rev. Genet. **5**:425–446.
78. **Holloway, B. W., and P. van de Putte.** 1968. Lysogeny and bacterial recombination, p. 175–183. *In* W. J. Peacock and R. D. Brock (ed.)., Replication and recombination of genetic material. Australian Academy of Sciences, Canberra.
79. **Holloway, B. W., and P. van de Putte.** 1968. Transducing phage for *Pseudomonas putida*. Nature (London) **217**:459–460.
80. **Holloway, B. W., H. Rossiter, D. Burgess, and J. Dodge.** 1974. Aeruginocin tolerant mutants of *Pseudomonas aeruginosa*. Genet. Res. **22**:239–253.
81. **Homma, J. Y., and H. Shionoya.** 1967. Relationship between pyocine and temperate phage of *Pseudomonas aeruginosa*. III. Serological relationship between pyocines and temperate phages. Jpn. J. Exp. Med. **37**:395–421.
82. **Hopwood, D. A., K. F. Chater, J. E. Dowding, and A. Vivian.** 1973. Advances in *Streptomyces coelicolor* genetics. Bacteriol. Rev. **37**: 371–405.
83. **van Hartingsveldt, J., and A. H. Stouthamer.** 1973. Mapping and characterization of mutants of *Pseudomonas aeruginosa* affected in nitrate

respiration in aerobic or anaerobic growth. J. Gen. Microbiol. **74**:97–106.

84. **Iglewski, B. H., and D. Kabat.** 1975. NAD-dependent inhibition of protein synthesis by *Pseudomonas aeruginosa* toxin. Proc. Natl. Acad. Sci. U.S.A. **72**:2284–2288.
85. **Iglewski, B. H., J. Sadoff, M. J. Bjorn, and E. S. Maxwell.** 1978. *Pseudomonas aeruginosa* exoenzyme S: an adenosine diphosphate ribosyltransferase distinct from toxin A. Proc. Natl. Acad. Sci. U.S.A. **75**:3211–3215.
86. **Ikeda, H., and J. Tomizawa.** 1968. Prophage P1, an extra-chromosomal replication unit. Cold Spring Harbor Symp. Quant. Biol. **33**: 791–798.
87. **Isaac, J. H., and B. W. Holloway.** 1968. Control of pyrimidine biosynthesis in *Pseudomonas aeruginosa*. J. Bacteriol. **96**:1732–1741.
88. **Jacob, A. E., J. M. Cresswell, and R. W. Hedges.** 1977. Molecular characterization of the P group plasmid R68 and variants with enhanced chromosome mobilizing ability. FEMS Microbiol. Lett. **1**:71–74.
89. **Jacob, F., and E. L. Wollman.** 1956. Sur les processes de conjugaison et de recominaison genetique chez *E. coli*. I. L'induction part conjugaison ou induction zygotique. Ann. Inst. Pasteur (Paris) **91**:486–510.
90. **Jacoby, G. A.** 1974. Properties of R plasmids determining gentamicin resistance by acetylation in *Pseudomonas aeruginosa*. Antimicrob. Agents Chemother. **6**:239–252.
91. **Jacoby, G. A.** 1979. Plasmids of *Pseudomonas aeruginosa*. *In* R. G. Doggett (ed.), *Pseudomonas aeruginosa*: clinical manifestations of infection and current therapy. Academic Press Inc., New York. (In press.)
92. **Jacoby, G. A., J. E. Rogers, A. E. Jacob, and R. W. Hedges.** 1978. Transposition of *Pseudomonas* toluene-degrading genes and expression in *Escherichia coli*. Nature (London) **274**: 179–180.
93. **Jacoby, G. A., and J. A. Shapiro.** 1977. Plasmids studied in *Pseudomonas aeruginosa* and other pseudomonads, p. 639–656. *In* A. I. Bukhari, J. A. Shapiro, and S. L. Adhya (ed.), DNA insertion elements, plasmids, and episomes. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
94. **Jarrell, K., and A. M. B. Kropinski.** 1976. The isolation and characterization of a lipopolysaccharide specific *Pseudomonas aeruginosa* bacteriophage. J. Gen. Virol. **33**:99–106.
95. **Jarrell, K., and A. M. Kropinski.** 1978. The chemical composition of the lipopolysaccharide from *Pseudomonas aeruginosa* strain PAO and a spontaneously derived rough mutant. Microbios **19**:103–116.
96. **Johnston, A. W. B., M. J. Bibb, and J. E. Beringer.** 1978. Tryptophan genes in Rhizobium—their organization and their transfer to other bacterial genera. Mol. Gen. Genet. **165**: 323–330.
97. **Johnston, J. B., and I. C. Gunsalus.** 1977. Isolation of metabolic plasmid DNA from *Pseudomonas putida*. Biochem. Biophys. Res. Commun. **75**:13–19.
98. **Kageyama, M.** 1970. Genetic mapping of a bacteriocinogenic factor in *Pseudomonas aeruginosa*. I. Mapping of pyocin R2 factor by conjugation. J. Gen. Appl. Microbiol. **16**:523–530.
99. **Kageyama, M.** 1970. Genetic mapping of a bacteriocinogenic factor in *Pseudomonas aeruginosa*. II. Mapping of pyocin R2 factor by transduction with phage F116. J. Gen. Appl. Microbiol. **16**:531–535.
100. **Kageyama, M.** 1974. Genetic mapping of pyocin R1 factor in *Pseudomonas aeruginosa*. J. Gen. Appl. Microbiol. **20**:269–275.
101. **Kageyama, M., and A. Inagaki.** 1974. Genetic mapping of pyocin R3 factor in *Pseudomonas aeruginosa*. J. Gen. Appl. Microbiol. **20**:257–267.

101a. **Kageyama, M., S. Yumiko, and T. Shinomiya.** 1979. Suppressor mutation in *Pseudomonas aeruginosa*. J. Bacteriol. **138**: (In press).

102. **Kemp, M. B., and G. D. Hegeman.** 1968. Genetic control of the β-ketoadipate pathway in *Pseudomonas aeruginosa*. J. Bacteriol. **96**: 1488–1499.
103. **Klingmuller, W., G. Buheitel, M. Filser, and A. Steiner.** Genetic manipulation of N_2-fixation in soil bacteria. *In* Proceedings of the Fourteenth International Genetics Congress, Moscow, August 1978. (In press.)
104. **Koval, S. F., and P. M. Meadow.** 1977. The isolation and characterisation of lipopolysaccharide defective mutants of *Pseudomonas aeruginosa* PAC 1. J. Gen. Microbiol. **98**:387–398.
105. **Krishnapillai, V.** 1971. A novel transducing phage. Its role in recognition of a possible new host-controlled modification system in *Pseudomonas aeruginosa*. Mol. Gen. Genet. **114**: 134–143.
106. **Krishnapillai, V., and K. E. Carey.** 1972. Chromosomal location of a prophage in *Pseudomonas aeruginosa* strain PAO. Genet. Res. **20**: 137–140.
107. **Kropinski, A. M. B., L. Chan, K. Jarrell, and F. H. Milazzo.** 1977. The nature of *Pseudomonas aeruginosa* strain PAO bacteriophage receptors. Can. J. Microbiol. **23**:656–658.
108. **Kropinski, A. M. B., L. Chan, and F. H. Milazzo.** 1978. Susceptibility of lipopolysaccharide-defective mutants of *Pseudomonas aeruginosa* PAO to dyes, detergents, and antibiotics. Antimicrob. Agents Chemother. **13**: 494–499.
109. **Lacy, G. H., and J. V. Leary.** 1976. Plasmid-mediated transmission of chromosomal genes in *Pseudomonas glycinea*. Genet. Res. **27**:363–368.
110. **Leidigh, B. J., and M. L. Wheelis.** 1973. The clustering on *Pseudomonas putida* chromosome of genes specifying dissimilatory functions. J. Mol. Evol. **2**:235–242.
111. **Leidigh, B. J., and M. L. Wheelis.** 1973. Genetic control of the histidine dissimilatory

pathway in *Pseudomonas putida*. Mol. Gen. Genet. **120**:201-210.

112. **Litchfield, J. H.** 1977. Comparative technical and economic aspects of single cell protein processes. Adv. Appl. Microbiol. **22**:267-305.
113. **Liu, P. V.** 1966. The roles of various fractions of *Pseudomonas aeruginosa* in its pathogenesis. II. Effects of lecithinase and protease. J. Infect. Dis. **116**:112-116.
114. **Loutit, J. S.** 1969. Investigation of the mating system of *Pseudomonas aeruginosa* strain 1. IV. Mapping of distal markers. Genet. Res. **13**: 91-98.
115. **Manoharan, T. H., and K. Jayaraman.** 1978. Mapping of the locus involved in the catabolic oxidation of D-amino acids in *Pseudomonas aeruginosa* PAO. Mol. Gen. Genet. **164**:51-56.
116. **Marinus, M. G., and J. S. Loutit.** 1969. Regulation of isoleucine plus valine biosynthesis in *Pseudomonas aeruginosa*. I. Characterization and mapping of mutants. Genetics **63**:547-556.
117. **Markowitz, S. M., F. L. Macrina, and P. V. Phibbs, Jr.** 1978. R-factor inheritance and plasmid content in mucoid *Pseudomonas aeruginosa*. Infect. Immun. **22**:530-539.
118. **Martin, D. R.** 1973. Mucoid variation in *Pseudomonas aeruginosa* induced by the action of phage. J. Med. Microbiol. **6**:111-118.
119. **Martinez, J., and P. H. Clarke.** 1975. R factor mediated gene transfer in *Pseudomonas putida*. Proc. Soc. Gen. Microbiol. **3**:51-52.
120. **Matsumoto, H., S. Ohta, R. Kobayashi, and Y. Terawaki.** 1978. Chromosomal location of genes participating in the degradation of purines in *Pseudomonas aeruginosa*. Mol. Gen. Genet. **167**:165-176.
121. **Matsumoto, H., and T. Tazaki.** 1973. FP5 factor, an undescribed sex factor of *Pseudomonas aeruginosa*. Jpn. J. Microbiol. **17**:409-417.
122. **Matsumoto, H., and T. Tazaki.** 1975. Serotypic recombination in *Pseudomonas aeruginosa*, p. 281-290. *In* S. Mitsuhashi and H. Hashimoto (ed.), Microbial drug resistance. University of Tokyo Press, Tokyo.
123. **Matsushiro, A., K. Sato, J. Ito, S. Kida, and F. Imamoto.** 1965. On the transcription of the tryptophan operon in *Escherichia coli*. J. Mol. Biol. **11**:54-63.
124. **Mee, B. J., and B. T. O. Lee.** 1967. An analysis of histidine requiring mutants in *Pseudomonas aeruginosa*. Genetics **55**:709-722.
125. **Mee, B. J., and B. T. O. Lee.** 1969. A map order for *HISI*, one of the genetic regions controlling histidine biosynthesis in *Pseudomonas aeruginosa*, using the transducing phage F116. Genetics **62**:687-696.
126. **Mergeay, M., A. Boyen, C. Legrain, and N. Glansdorff.** 1978. Expression of *Escherichia coli* K-12 arginine genes in *Pseudomonas fluorescens*. J. Bacteriol. **136**:1187-1188.
128. **Mergeay, M., and J. Gerits.** 1978. F'-plasmid transfer from *Escherichia coli* to *Pseudomonas fluorescens*. J. Bacteriol. **135**:18-28.
129. **Miller, R. V., and C.-H. C. Ku.** 1978. Characterization of *Pseudomonas aeruginosa* mutants deficient in the establishment of lysogeny. J. Bacteriol. **134**:875-883.
130. **Miller, R. V., J. M. Pemberton, and A. J. Clark.** 1977. Prophage F116: evidence for extrachromosomal location in *Pseudomonas aeruginosa* strain PAO. J. Virol. **22**:844-847.
131. **Mills, B. J., and B. W. Holloway.** 1976. Mutants of *Pseudomonas aeruginosa* that show specific hypersensitivity to aminoglycosides. Antimicrob. Agents Chemother. **10**:411-416.
132. **Mindich, L., J. Cohen, and M. Weisburd.** 1976. Isolation of nonsense suppressor mutants in *Pseudomonas*. J. Bacteriol. **126**:177-182.
133. **Minina, T. S., E. A. Andreevskaya, and I. V. Domaradskÿ.** 1978. New indications on the participation of plasmid R of group p in the transfer of chromosomal genes in intergenera crosses. J. Microbiol. Epidemiol. Immunobiol. U.S.S.R. **13**:61-66.
134. **Moillo, A. M.** 1973. Isolation of a transducing phage forming plaques on *Pseudomonas maltophilia* and *Pseudomonas aeruginosa*. Genet. Res. **21**:287-289.

134a. **Morgan, A. F.** 1979. Transduction of *Pseudomonas aeruginosa* with a mutant of bacteriophage E79. J. Bacteriol. (submitted).

135. **Morriss, J. E., and H. E. Heath.** 1979. Structure of the *trp* gene clusters in *Pseudomonas acidovorans*. J. Bacteriol. (submitted).
136. **Mylroie, J. R., D. A. Friello, and A. M. Chakrabarty.** 1978. Transformation of *Pseudomonas putida* with chromosomal DNA. Biochem. Biophys. Res. Commun. **82**:281-288.
137. **Mylroie, J. R., D. A. Friello, T. V. Siemens, and A. M. Chakrabarty.** 1977. Mapping of *Pseudomonas putida* chromosomal genes with a recombinant sex-factor plasmid. Mol. Gen. Genet. **157**:231-237.
138. **Nagahari, K., and K. Sakaguchi.** 1978. RSF1010 plasmid as a potentially useful vector in *Pseudomonas* species. J. Bacteriol. **133**: 1527-1529.
139. **Nagahari, K., T. Tanaka, F. Hishinuma, M. Kuroda, and K. Sakaguchi.** 1977. Control of tryptophan synthetase amplified by varying the number of composite plasmids in *E. coli* cells. Gene **1**:141-152.
140. **Nagahari, K., Y. Sano, and K. Sakaguchi.** 1977. Derepression of *E. coli trp* operon on interfamilial transfer. Nature (London) **266**: 745-746.
141. **Nakazawa, T.** 1968. TOL plasmid in *Pseudomonas aeruginosa* PAO: thermosensitivity of self-maintenance and inhibition of host cell growth. J. Bacteriol. **133**:527-535.
142. **Nakazawa, T., E. Hayashi, T. Yokota, Y. Ebina, and A. Nakazawa.** 1978. Isolation of TOL and RP4 recombinants by integrative suppression. J. Bacteriol. **134**:270-277.
143. **Oishi, M., and S. D. Cosloy.** 1972. The genetic and biochemical basis of the transformability of *Escherichi coli* K12. Biochem. Biophys. Res. Commun. **49**:1568-1572.
144. **Olsen, R. H., and J. Hansen.** 1976. Evolution and utility of a *Pseudomonas aeruginosa* drug

resistance factor. J. Bacteriol. **125**:837–844.
145. **Olsen, R. H., and E. S. Metcalf.** 1968. Conversion of mesophilic to psychrophilic bacteria. Science **162**:1288–1289.
146. **Olsen, R. H., E. S. Metcalf, and J. K. Todd.** 1968. Characteristics of bacteriophages attacking psychrophilic and mesophilic pseudomonads. J. Virol. **2**:357–364.
147. **Palchaudhuri, S.** 1977. Molecular characterization of hydrocarbon degradative plasmids in *Pseudomonas putida*. Biochem. Biophys. Res. Commun. **77**:518–525.
148. **Pate, R. E., L. M. Smith, and H. E. Heath.** 1979. Organization of the *hut* genes in *Pseudomonas acidovorans*. J. Bacteriol. (submitted).
149. **Patel, R. N., and L. N. Ornston.** 1976. Immunological comparison of enzymes of the β-ketoadipate pathway. Arch. Mikrobiol. **110**:27–36.
150. **Pemberton, J. M.** 1974. Size of the chromosome of *Pseudomonas aeruginosa* PAO. J. Bacteriol. **119**:748–752.
151. **Pemberton, J. M., and B. W. Holloway.** 1972. Chromosome mapping in *Pseudomonas aeruginosa*. Genet. Res. **19**:251–260.
152. **Phibbs, P. V., Jr., S. M. McCowen, T. W. Feary, and W. T. Blevins.** 1978. Mannitol and fructose catabolic pathways of *Pseudomonas aeruginosa* carbohydrate-negative mutants and pleiotropic effects of certain enzyme deficiencies. J. Bacteriol. **133**:717–728.
153. **Ravin, V. K., and M. G. Shulga.** 1970. Evidence for extrachromosomal location of prophage N15. Virology **40**:800–807.
154. **Reynold, H. Y., A. S. Levine, R. E. Wood, C. H. Zierdt, D. C. Dale, and J. E. Pennington.** 1975. *Pseudomonas aeruginosa* infections: persisting problems and current research to find new therapies. Ann. Intern. Med. **82**:819–831.
155. **Riley, M., L. Solomon, and D. Zipkas.** 1978. Relationship between gene function and gene location in *Escherichia coli*. J. Mol. Evol. **11**:47–56.
156. **Rosenberg, S. L., and G. D. Hegeman.** 1969. Clustering of functionally related genes in *Pseudomonas aeruginosa*. J. Bacteriol. **99**:353–355.
157. **Sanderson, K. E., and P. E. Hartman.** 1978. Linkage map of *Salmonella typhimurium*, edition V. Microbiol. Rev. **42**:471–519.
158. **Sano, Y., and M. Kageyama.** 1977. Transformation of *Pseudomonas aeruginosa* by plasmid DNA. J. Gen. Appl. Microbiol. **23**:183–186.
159. **Schmieger, H.** 1971. A method for detection of phage mutants with altered transducing ability. Mol. Gen. Genet. **110**:378–831.
160. **Schmieger, H.** 1972. Phage P22 mutants with increased or decreased transduction abilities. Mol. Gen. Genet. **119**:75–88.
161. **Shaham, M., A. M. Chakrabarty, and I. C. Gunsalus.** 1973. Camphor plasmid-mediated chromosomal transfer in *Pseudomonas putida*. J. Bacteriol. **116**:944–949.
162. **Shinomiya, T., M. Ohsumi, and M. Kageyama.** 1975. Defective pyocin particles produced by some mutant strains of *Pseudomonas aeruginosa*. J. Bacteriol. **124**:1508–1521.
163. **Sinclair, M. I., and A. F. Morgan.** 1978. Transformation of *Pseudomonas aeruginosa* strain PAO with bacteriophage and plasmid DNA. Aust. J. Biol. Sci. **31**:679–688.
164. **Smyth, P. F., and P. H. Clarke.** 1975. Catabolite repression of *Pseudomonas aeruginosa* amidase: isolation of promoter mutants. J. Gen. Microbiol. **90**:91–99.
165. **Sobieski, R. J., and R. H. Olsen.** 1973. Cold-sensitive *Pseudomonas* RNA polymerase. II. Cold-promoted restriction of bacteriophage CB3 and the lack of host-dependent bacteriophage-specific RNA transcription. J. Virol. **12**:1384–1394.
166. **Stanier, R. Y., and L. N. Ornston.** 1973. The β-ketoadipate pathway. Adv. Microbial Physiol. **9**:89–151.
167. **Stanisich, V. A.** 1976. Isolation and characterization of plasmids in *Pseudomonas aeruginosa*. Bull. Inst. Pasteur (Paris) **74**:285–294.
168. **Stanisich, V. A., P. M. Bennett, and M. H. Richmond.** 1977. Characterization of a translocation unit encoding resistance to mercuric ions that occurs on a nonconjugative plasmid in *Pseudomonas aeruginosa*. J. Bacteriol. **129**:1227–1233.
169. **Stanisich, V. A., and B. W. Holloway.** 1969. Genetic effects of acridines on *Pseudomonas aeruginosa*. Genet. Res. **13**:57–70.
170. **Stanisich, V. A., and B. W. Holloway.** 1971. Chromosome transfer in *Pseudomonas aeruginosa* mediated by R factor. Genet. Res. **17**:169–172.
171. **Stanisich, V. A., and B. W. Holloway.** 1972. A mutant sex factor of *Pseudomonas aeruginosa*. Genet. Res. **19**:91–108.
172. **Stanisich, V. A., and M. H. Richmond.** 1975. Gene transfer in the genus *Pseudomonas*, p. 163–190. *In* P. H. Clarke and M. H. Richmond (ed.), Genetics and biochemistry of pseudomonas. J. Wiley & Sons, London.
173. **Stocker, B. A. D., and P. H. Mäkelä.** 1971. Genetic aspects of biosynthesis and structure of *Salmonella* lipopolysaccharide, p. 369–438. *In* G. Weinbaum, S. Kadis, and S. J. Ajl (ed.), Microbial toxins, vol. 4. Academic Press Inc., New York.
174. **Thacker, R., O. Rorvig, P. Kahlon, and I. C. Gunsalus.** 1978. NIC, a conjugative nicotine-nicotinate degradative plasmid in *Pseudomonas convexa*. J. Bacteriol. **135**:289–290.
175. **Thurm, P., and A. J. Garro.** 1975. Isolation and characterization of prophage mutants of the defective *Bacillus subtilis* bacteriophage PBSX. J. Virol. **16**:184–191.
176. **de Torrontegui, G., R. Diaz, M. C. Wheelis, and J. L. Canavos.** 1976. Supraoperonic clustering of genes specifying glucose dissimilation in *Pseudomonas putida*. Mol. Gen. Genet. **44**:307–311.
177. **Tucker, W. T., and J. M. Pemberton.** 1978.

Viral R plasmid Rφ6P: properties of the penicillinase plasmid prophage and the supercoiled, circular encapsidated genome. J. Bacteriol. **135**:207–214.

178. **Vincente, M., M. A. de Pedro, G. de Torrontegui, and J. L. Cànovas.** 1975. The uptake of glucose and gluconate by *Pseudomonas putida*. Mol. Cell. Biochem. **7**:59–64.
179. **Voellmy, R., and T. Leisinger.** 1975. Dual role for N^2-acetylornithine 5-aminotransferase from *Pseudomonas aeruginosa* in arginine biosynthesis and arginine catabolism. J. Bacteriol. **122**:799–809.
180. **Watson, J. M., and B. W. Holloway.** 1976. Suppressor mutations in *Pseudomonas aeruginosa*. J. Bacteriol. **125**:780–786.
181. **Watson, J. M., and B. W. Holloway.** 1978. Chromosome mapping in *Pseudomonas aeruginosa* PAT. J. Bacteriol. **133**:1113–1125.
182. **Watson, J. M., and B. W. Holloway.** 1978. Linkage map of *Pseudomonas aeruginosa* PAT. J. Bacteriol. **136**:507–521.
183. **Watson, M. D., and J. G. Scaife.** 1978. Chromosomal transfer promoted by the promiscuous plasmid RP4. Plasmid **1**:226–237.
184. **Wheelis, M. L.** 1975. The genetics of dissimilatory pathways in *Pseudomonas*. Annu. Rev. Microbiol. **29**:505–524.
185. **Wheelis, M. L., and R. Y. Stanier.** 1970. The genetic control of dissimilatory pathways in *Pseudomonas putida*. Genetics **66**:245–266.
186. **Woods, W. H., and J. B. Egan.** 1974. Prophage induction of noninducible coliphage 186. J. Virol. **14**:1349–1356.
187. **Wretlind, B., L. Sjöberg, and T. Wadström.** 1977. Protease-deficient mutants of *Pseudomonas aeruginosa*: pleiotropic changes in activity of other extracellular enzymes. J. Gen. Microbiol. **103**:329–336.
188. **Wretlind, B., and T. Wadström.** 1977. Purification and properties of a protease with elastase activity from *Pseudomonas aeruginosa*. J. Gen. Microbiol. **103**:319–327.
189. **Wu, T. T.** 1966. A model for three-point analysis of random general transduction. Genetics **54**: 405–410.
190. **Yarmolinsky, M. B.** 1977. Genetic and physical structure of bacteriophage P1 DNA, p. 721–732. *In* A. I. Bukari, J. A. Shapiro, and S. L. Adhya (ed.), DNA insertion elements, plasmids, and episomes. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
191. **Yeh, W. K., G. Davis, P. Fletcher, and L. N. Ornston.** 1978. Homologous amino acid sequence in enzymes mediating sequential metabolic reactions. J. Biol. Chem. **252**:4920–4923.
192. **Young, F. E., and G. A. Wilson.** 1974. *Bacillus subtilis*, p. 69–114. *In* R. C. King (ed.), Handbook of genetics, vol. 1, Bacteria, bacteriophages and fungi. Plenum Press, New York.