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Genetics of *Pseudomonas*

B. W. HOLLOWAY

Department of Genetics, Monash University, Clayton, Victoria, 3168, Australia

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INTRODUCTION

The genus *Pseudomonas* has many characteristics which make it a suitable subject for genetic analysis. For many years, various species of this genus have been extensively studied for their metabolic properties, revealing a group of organisms with considerable biochemical interest. One species, *P. aeruginosa*, is associated with a variety of human disease conditions, and many other species can be readily isolated from soil and water. The general taxonomic and biochemical characteristics of the pseudomonads have recently been described in some detail (33, 128).

Until recently, most of the genetic work on this genus has been carried out by using *P. aeruginosa*. Historically, the selection of this species was made at a time when very little was known about genetic systems in bacteria other than *Escherichia coli*, *Salmonella typhimurium*, and the pneumococci. *P. aeruginosa* has many characteristics

which make it a suitable choice for combined biochemical and genetic study; it does not have stringent growth requirements and will grow on most of the common bacteriological media, including a chemically defined minimal medium (137). As it is associated with a variety of human disease conditions, many different strains are usually available from any hospital, although it does not appear to act as a pathogen under laboratory conditions and, hence, stringent precautions to prevent laboratory infection are not required. This species is highly resistant to many deleterious agents, and, while not possessing the same range of metabolic activities of, say, *P. putida*, it possesses a pattern of metabolism quite different from that of the *Enterobacteriaceae*. As *P. aeruginosa* is frequently lysogenic, bacteriophages are available as a concomitant genetic tool.

The other species which has attracted genetic attention is *P. putida*, a saprophytic species, taxonomically distinct from *P. aeruginosa*. It can be readily isolated from soil, and its ability to utilize a large variety of carbon and nitrogen sources has been extensively studied. Until quite recently, little was known of its associated bacteriophages.

It is the purpose of this review to assess the current state of genetic knowledge of *Pseudomonas* and its bacteriophages, to point out where such studies have revealed differences from other bacteria, particularly the enterobacteria, and to discuss the value of the *Pseudomonas* material for the study of particular genetic problems.

AVAILABILITY OF MUTANTS IN PSEUDOMONAS

The types of mutants available in a given organism very often determine its genetic usefulness. *Pseudomonas* has both advantages and disadvantages in this respect. In view of the usual techniques for recombinant selection in bacteria first introduced by Lederberg and Tatum (84), the availability of auxotrophs is a distinct advantage, and, with modification of the standard techniques, this class of mutant can be readily isolated in *Pseudomonas*. A recent technique for mutant isolation in *Pseudomonas* uses repeated counterselection of wild-type cells in the presence of D-cycloserine and penicillin (114).

Other mutants available include those exhibiting drug and phage resistance, and temperature sensitivity. *P. aeruginosa* is insensitive to many metabolite analogues (139), but it is sensitive to *p-fluorophenylanine* (FPA), and mutants resistant to FPA (FPA-r) have been isolated and studied in both *P. aeruginosa* and *P. putida*. In view of the wide metabolic versatility of *Pseudomonas*, many mutants with altered ability to use a wide range of carbon or nitrogen sources are potentially available. This may apply to mutants which have lost the ability to use a particular carbon source, for example β-ketoadipic acid (79), or have acquired the ability to use a new substrate, such as in *P. putida* where a mutant able to use D-camphor as sole carbon source has been isolated (22). Such mutants, being unavailable in many other bacteria, provide a unique opportunity for biochemical genetic analysis in *Pseudomonas*. Although one of the diagnostic features of *Pseudomonas* is its production of water-soluble pigments, no genetic analysis of their production has yet been made, nor have they been used for genetic markers. Pigmentation variants of *P. aeruginosa* were studied by Howarth and Dedman (74).

A feature of the in vitro growth of *P. aeruginosa* is the formation of cultural variants, and reports on this dissociation, as is known, date back to some of the earliest publications on *P. aeruginosa*. They refer to changes in colonial morphology (mucoid or nonmucoid, smooth or rough), pigmentation, irridescence, and others (46). A more recent study (144) showed that the ability to dissociate in this way is not a property of all strains and in addition can occur both in vivo and in artificial culture. Such colonial variation also occurs in *P. putida* (22) but has not been so extensively studied. In *P. aeruginosa* this variation has two important consequences. First, it means that characteristics such as pigmentation which show this dissociation are not particularly suitable as genetic markers. Second, the dissociants often show alterations from the parent culture in phage susceptibility, thus creating difficulties in phage typing for epidemiological studies and in transduction studies.

BACTERIOPHAGES OF PSEUDOMONAS

It is convenient to first discuss bacteriophage studies in *Pseudomonas*, not only because transduction is one of the important mechanisms of genetic analysis in this genus, but because the study of *Pseudomonas* bacteriophages has provided other information of genetic interest. Those bacteriophages and bacterial strains which have been extensively used for genetic research are listed in Table 1.

There is quite an extensive literature on bacteriophages of this genus. Until quite recently, this literature was confined to *P. aeruginosa* and the plant pathogenic species, but bacteriophages for *P. putida* and *P. fluorescens* have now been isolated and studied. Phages may be isolated from lysogenic strains, from sewage, or from soil.

TABLE 1. *Bacteriophages and bacterial strains of Pseudomonas commonly used in genetic studies*

Strain	Description	References
Bacterial strains		
P. aeruginosa PAO1	Wild-type strain formerly numbered 1C; other PAO strains are derived from this parent strain (ATCC15692)	57
P. aeruginosa PAT2	Wild-type strain, formerly numbered 2 (ATCC15693)	38, 57
P. putida PUG1	Formerly C1B, wild type	20, 22
P. putida PUG2	Formerly C1S, wild type	20, 22
P. putida PRS1	Formerly A3.12, wild type	128
Bacteriophage strains		
P. aeruginosa B3	Temperate noninducible, transducing, shows HCM; mol wt of DNA, ca. 20×10^6 daltons ATCC 15692-B	70, 126
P. aeruginosa D3	Temperate, inducible, nontransducing, shows phage conversion, shows HCM	63, 70
P. aeruginosa E79	Intemperate, nontransducing, does not show HCM	70
P. aeruginosa F116	Temperate, inducible, transducing; mol wt of DNA, ca. 35–40 $\times 10^6$ daltons, does not show HCM	64, 70
P. aeruginosa G101	Temperate, noninducible, transducing, shows HCM	69
P. aeruginosa B	Temperate, transducing	116
P. putida pf10	Nonlysogenizing	108
P. putida pf15	Nonlysogenizing, recombination studies	23, 108
P. putida pf16	Transducing, nonlysogenizing	22, 108

General Nature of Pseudomonas Phages

In terms of morphology, the range of phage types isolated for *Pseudomonas* appears to be as great as that for other bacteria. Bradley (14) has listed for *Pseudomonas* all the known morphological types with the exception of the tailless phages with large capsomeres. A number of electron-micrograph studies of *Pseudomonas* phages have been made (12–14, 40, 126, 133). Ribonucleic acid (RNA) phages have been isolated for *P. aeruginosa* but, unlike most of the *E. coli* RNA phages, they are sex-indifferent—plating equally well on male and female strains and showing adsorption characteristics different from those of comparable phages in *E. coli* (19, 39, 40). Whereas, in general, the host range of *Pseudomonas* phages seems to follow species lines, some phages can infect a range of different species. For example, phage Px14 can infect *P. geniculata*, *P. fluorescens*, and *P. aeruginosa* (109). A group of phages isolated from sewage (112) were found to have varying host ranges depending on the species used for their isolation. Those isolated on *P. aeruginosa* plated only on that species, but others isolated on *P. putida* and *P. fluorescens* not only plated on those two species but also on *P. aeruginosa* and some soil pseudomonads.

Recently, bacteriophages active on *P. putida* C1, a strain used extensively in studies on terpene metabolism, have been isolated from sewage (108) and characterized with respect to morphology, serological relationship, host range, and nucleic acid. One of these phages, pf16, is capable of general transduction (*see below*).

Infection of *Pseudomonas* with a quite unrelated phage has been reported (2) in that the coliphage P1 will attach to and kill *P. aeruginosa*. P1 does not multiply in *P. aeruginosa* with the production of mature phage, but there is some indication that the phage deoxyribonucleic acid (DNA) enters the bacterium.

Lysogeny

The frequency of occurrence of lysogeny in different species of *Pseudomonas* is highly variable. Lysogeny of naturally occurring isolates of *P. putida* has not been reported. Patterson (115) has reported three strains of *P. fluorescens* to be lysogenic, and lysogeny in plant pathogenic species of *Pseudomonas* has been described (42).

By contrast, most strains of *P. aeruginosa* are lysogenic for at least one phage, and the occurrence of more than one prophage type in a lysogenic strain is quite common (59, 70, 115, 144). The high frequency of lysogeny and aeruginocinogenicity in *P. aeruginosa* has enabled a typing method to be devised for epidemiologically important strains of this species (44, 59).

It is likely that the real frequency of lysogeny and multiple lysogeny reported by most workers in this species is an underestimate, owing to the frequency with which differences in DNA specificity occur with different strains of *P. aeruginosa*. Many, but not all, phages of this species show host-controlled modification (HCM; *see below*). When temperate phage particles are released by lysis from a given lysogenic bacterial strain, they acquire the host specificity of that strain. When

plated on an indicator strain used to detect lysogeny, as in the Fisk method (41), the phage released may be restricted by the indicator strain, thus reducing its plating efficiency, often by a factor of 10^{-5}. As the supernatant fluids of broth cultures of lysogenic *Pseudomonas* strains usually contain between 10^2 and 10^6 free phage particles per ml, the test strain could be falsely scored as nonlysogenic. By using restriction-deficient bacterial strains (*see* section on HCM) in procedures to detect lysogeny, such difficulties may be overcome, and this is shown both by an increase in the number of lysogenic strains detected and also by the number of such strains which release more than one type of bacteriophage (Holloway, *unpublished observations*).

Temperate phages of *Pseudomonas* are either inducible or noninducible, and induction can occur by ultraviolet irradiation (70, 105) or by such chemicals as mitomycin C (143) or methyl-nitro-nitroso-guanidine (van de Putte, *unpublished observations*).

Genetic Control of Lysogeny

The genetic control of lysogeny in *P. aeruginosa* appears to involve the genome of both the phage and the bacterium. Egan and Holloway (36) identified loci of the phage D3 (*see* Table 1) affecting lysogenization, the situation being essentially similar to that of coliphage lambda (78) or the *S. typhimurium* phage P22 (88). There are three closely linked loci in D3 which affect lysogenization frequency, as indicated by the isolation of clear plaque mutants which show complementation with each other.

The role of the bacterial genome in lysogenization has also been considered. It is clear that the genetic control of integration by prophages such as λ or φ80 in *E. coli* resides in the bacteriophage genome (124). This means that the bacterial genome has no effect on the establishment or persistence of lysogeny, and hence it should not be possible to isolate bacterial mutants which affect these properties. However, in *P. aeruginosa* such mutants have been isolated (62, 136) by cross-streaking the survivors of mutagenic treatment against a temperate phage. Some surviving clones show a greater lytic response to the temperaet phage and give clearer plaques with such phages than when these phages are plated onto wild-type bacteria. Such lysogenic deficient mutants (*les*; changes in lysogenic establishment) show a similar response to all temperate phages so far tested, including B3, D3, F116, and G101. It is possible to lysogenize such *les* mutants, but the frequency of lysogenization is very dependent upon the multiplicity of infection and is generally negligible below a multiplicity of infection of three, although the actual figure varies with different phages and different mutants. At higher multiplicites of infection, the lysogenization frequency approaches that of the wild type. When lysogenized with an inducible phage, such lysogenic strains of *les* mutants cannot be induced by ultraviolet (UV) radiation. It may be that in *Pseudomonas* the activity of the phage integration mechanism is inefficient, and hence bacterial recombination mechanisms are required for lysogeny to occur. Thus in the wild type (*les*$^+$), the bacterial recombination system is involved in the integration of prophage, whereas in the mutant (*les*), with the absence of the bacterial recombination system, the presence of a number of phage genomes is required to provide enough integration enzyme for the prophage to be inserted into the bacterial chromosome. An alternative hypothesis is that integration may be related to the level of phage repressor, and the production and maintenance of this repressor is affected by both the bacterial and phage genomes. In either case, it can be said that both phage and bacterial genome contribute to the establishment of lysogeny in *P. aeruginosa*.

If the phage integration system is secondary to the bacterial recombination system, it follows that bacterial mutants lacking the ability to be lysogenized may be recombination deficient (Rec$^-$). In fact, Les$^-$ isolates are frequently Rec$^-$; their properties are described in a later section.

This relationship between lysogenization potential and recombination ability is also shown by other bacteria. A search for mutants of the Les$^-$ type in *E. coli* K-12, with lambda as the screening phage, was not successful (24), but Sironi has isolated mutants of *E. coli* C which have a reduced ability to be both lysogenized by phage P2 and transduced by phage P1 (125). A recombination-deficient mutant of *S. typhimurium* integrates phage P22 strain at a frequency of 10% of the wild-type bacterial strain (140). It is found that mutants with a Rec$^-$ phenotype of *P. aeruginosa*, *E. coli*, and *S. typhimurium* show an additional common response in that, when lysogenized with an inducible phage (for example, D3 for *P. aeruginosa*, λ for *E. coli*, and P22 for *S. typhimurium*) such Rec$^-$ lysogens are not inducible by UV.

Phage Conversion

The phenomenon of phage conversion (5), well known in other phage-bacterial systems, has been reported for *P. aeruginosa* (63). When the temperate phage D3 lysogenizes *P. aeruginosa* strain PA01, a new surface antigen is produced, such that D3 now cannot adsorb to this lysogenic strain.

In a slightly different case, it has been found that, when *P. aeruginosa* PAO1 is made lysogenic for phage F116, the plating efficiency of the unrelated phage B3 is greatly reduced (118). This is not due to changes in adsorption, nor is it due to HCM. It appears to be the result of the suppression of multiplication of the unrelated phage by the F116 prophage, an effect similar to that occurring for bacteriophage BF23 in *E. coli* strains colicinogenic for colicin I (131).

It is to be expected that, in an organism such as *P. aeruginosa* in which lysogeny is such a common characteristic, interactions of this type will not only be found but that they will constitute an important aspect of the genetic potential of the bacterium. The contribution of phage genetic material to the bacterial genome may be more common than is generally recognized. For example, it is known in other bacteria that prophages may affect DNA specificity (HCM; 85). It may be worthwhile to look for other changes, including effects on bacterial recombination mechanisms, which are the result of prophage establishment.

Recombination in Pseudomonas Phages

This has been demonstrated for both *P. aeruginosa* phage D3 (36) and *P. putida* phage pf15 (23). Preliminary mapping of the phage chromosome has been done in each case, the mutants used being mainly plaque morphology mutants. In view of the lack of clustering of related genes demonstrated in the mapping of the *Pseudomonas* bacterial chromosome (*see below*), it would be interesting to carry out additional mapping of these phages to see whether this type of gene arrangement extends to the bacteriophage chromosome, and thus differs from the situation in the coliphages lambda and T4.

HOST-CONTROLLED MODIFICATION (HCM)

Nature of HCM

It is now known from studies on a variety of bacteria that individual bacterial strains show a DNA specificity. This can be demonstrated either by HCM of bacteriophage or by effects on bacterial recombination in either transduction or conjugation. In HCM, the plating ability of a bacteriophage for various bacterial strains is dependent upon the bacterial strain on which it was most recently propagated; and this has been extensively studied in coliphages such as lambda (4). The way in which HCM affects plating efficiency is shown in Table 2.

TABLE 2. *HCM of phage B3 on three unrelated strains of P. aeruginosa: PAO1, PAT2, and PTS271*

Phage	Plating efficiency on		
	PAO1	PAT2	PTS271
B3 propagated on PAO1 (B3.1)	1.0	10^{-5}	10^{-5}
B3 propagated on PAT2 (B3.2)	10^{-5}	1.0	10^{-5}
B3 propagated on PTS271 (B3.271)	10^{-5}	10^{-5}	1.0

HCM involves two separate and distinguishable processes. (i) In *restriction*, bacteriophage, plasmid, or bacterial DNA, when introduced into a bacterial cell, is recognized as acceptable, thus enabling the normal biological functions of the DNA to proceed, or unacceptable, when the DNA is degraded enzymatically, with consequent loss of biological function. (ii) In the process of *modification*, the bacterium imposes a specificity on any DNA, bacterial or episomal, which is synthesized by the bacterium. Restriction is the mechanism by which this specificity is recognized.

The chemical basis of this DNA specificity is not yet generally established. However, in one case, that of the restriction of T-even phages by certain *E. coli* strains, it has been shown to be due to the glucosylation of the 5-hydroxymethylcytosine of the phage DNA. In other cases, there is some evidence that methylation of the DNA may be involved (4, 56).

HCM can be readily demonstrated in *P. aeruginosa*. Its occurrence depends both upon the bacterial strains and the bacteriophage involved. It has not been reported in other species of *Pseudomonas*. The general nature of HCM in *P. aeruginosa* is the same as that found in *E. coli* or *S. typhimurium* with respect to enzymatic degradation of host-modified bacteriophage DNA, multiplicity activation (135), heat inactivation of restriction, and modification changes following single-cycle infection of a modifying host (118).

Genetic Control of HCM in P. aeruginosa

A genetic analysis of the control of HCM in *P. aeruginosa* has revealed a number of differences from the situation in *E. coli*. One feature is that, as yet, no episome such as a prophage has been shown to be responsible for these DNA specificity differences, unlike the situation with phage P1 or R factors in *E. coli*. The genetic control of HCM in *P. aeruginosa* is complex and may be classified under the following headings.

Gene differences between strains. As will be

described in more detail in a subsequent section, conjugation has been demonstrated in *P. aeruginosa*. Two unrelated strains of *P. aeruginosa*, PAO1 and PAT2 (for details of these strains *see* Table 1), were shown to be interfertile. Certain phages, for example B3, show a reciprocal or two-way HCM when propagated in turn upon these two parent strains (Table 2). As the determination of host specificity is a bacterial function, it can be concluded that PAO1 and PAT2 differ genetically in this respect. This has been confirmed by crosses between auxotrophic derivatives of PAO1 and PAT2 (67, 118) which showed segregation of genes affecting HCM. These crosses indicated that control of HCM differences between these two strains is vested in more than one gene, and that the pattern of segregation of these genes indicates that the genes involved are not linked. Not only can recombinants be isolated which have HCM properties unlike either parent, but clearly the chromosomal distribution of these HCM genes is quite unlike that of *E. coli*, which appears to have a cluster of HCM genes all closely linked in the *thr-leu* region (4). Detailed linkage analysis of these genes in *P. aeruginosa* was not possible because, in these crosses between PAO1 and PAT2, the restriction mechanisms act on bacterial DNA. The chromosome of PAT2, on entering strain PAO1, is broken down by restriction enzymes, obscuring the segregation patterns of the donor genes. It follows that any more intensive mapping or identification of these HCM loci becomes somewhat unreliable unless crosses can be carried out with females lacking restriction.

Induced mutations affecting HCM. In further studies, however, it was shown that other loci can affect the expression of HCM. In the inter-strain differences just described, the gene differences were those occurring naturally between strains PAO1 and PAT2. However, it is found that restriction (Res) and modification (Mod) mutants can be readily isolated from PAO1, and mutants of the Res^- Mod^+, Res' Mod^+, Res^- Mod', and Res^- Mod^- phenotypes have been identified, the prime (′) types having a less extreme phenotypic change than the negative (−) types (118, 120). Crosses between these mutants and strain PAT2 indicated that the chromosomal locations of at least some of these mutations are not the same as the markers responsible for HCM differences between strains PAO1 and PAT2, and it can be concluded that a number of different loci, perhaps more than six, are concerned with the control of HCM in *P. aeruginosa* (118). However, this is not the complete story of the genetic basis of HCM in this organism.

Phenotypic changes in HCM caused by growth of bacteria at 43 C. When some, but not all, strains of *P. aeruginosa* (for example PAO1 and PTS271 but not PAT2) are grown at 43 C, alterations in the HCM phenotype result (60). Such 43 C-grown bacteria cannot distinguish between bacteriophages of different host specificity and hence are phenotypically Res^-. Table 3 illustrates the observed effects on plating efficiency. In itself this would not be so unusual, except for the fact that this loss of restriction (and concomitant changes in modification properties) persists for about 60 generations after return of the 43 C-grown culture to growth at 37 C. The phenomenon (which will be referred to as the 43 C effect) can only be induced by growth, not merely exposure, at 43 C, and as few as five generations at 43 C is all that is required to induce it. The persistence of the effect at 37 C after growth at 43 C is completely independent of how the culture is grown at 37 C, variations in type of medium, aeration, inoculum, size of transfer, etc., having no effect on the number of generations of persistence. When the HCM phenotype does return to that characteristic for 37 C, it may be as readily returned to the 43 C phenotype by growth at that temperature as if the culture had never been previously exposed to the higher temperature.

The 43 C effect can only be induced when the rate of oxygen supply is limiting. *P. aeruginosa* is an obligate aerobe, so that some degree of aeration is necessary, but with excess aeration at 43 C, obtained by shaking the cultures or by passing air through a sparger into the culture, there is no change in the HCM properties from the 37 C phenotype.

Any explanation of these facts must take into account the following considerations. (i) Apart from HCM, no other aspects of the bacterial phenotype are affected in this way by growth at 43 C. (ii) Reversion from the 43 C phenotype to the 37 C phenotype for HCM always occurs. Furthermore, such reverted cells can be made to

TABLE 3. *Effect on plating efficiency (free phage assays) of host-modified phages*[a]

Bacteriophage	Bacterial strain			
	PAO1 (grown at 37 C)	PAO1 (grown at 43 C)	PTS271 (grown at 37 C)	PTS271 (grown at 43 C)
B3.1	1.0	1.0	10^{-5}	1.0
B3.271	10^{-5}	1.0	1.0	1.0

[a] Indicator strains of *P. aeruginosa* were pregrown at 37 or 43 C before being used as indicator strains. All plaque counts were incubated at 37 C.

show the 43 C effect in exactly the same way as if they had never been grown at 43 C before.

This latter feature suggests a conservation of genetic information and, hence, any explanation of the 43 C effect involving mutation or the selection of mutants would be unlikely to have the required specificity. Experiments to implicate a plasmid in this phenomenon have been unsuccessful. The most likely explanation would seem to involve some steady-state hypothesis involving a differential rate of synthesis at 37 and 43 C of either HCM enzymes or a repressor controlling such enzymes. It can be suggested that growth at 43 C alters the rate of synthesis of a protein such that the HCM phenotype characteristic of 37 C-grown cells is changed. When such 43 C-grown bacteria are returned to 37 C, it requires many generations at the lower temperature to return to the steady-state situation producing the 37 C HCM phenotype. A possibly analogous situation involving such a persistence of phenotype was recently described in *E. coli* K-12 (37).

Pleiotropic effects of other mutations on HCM. It is generally known that mutations to streptomycin resistance or dependence can affect a variety of phenotypic properties including HCM in both *E. coli* (35, 85) and *P. aeruginosa* (67). Another type of mutation has been found to have extensive effects on HCM in *P. aeruginosa*, namely, resistance to FPA. It was found, fortuitously, that over 50% of the FPA-r mutants tested showed concomitant alterations in HCM phenotype (120). Further work on the mechanism of FPA resistance (138; Dunn and Holloway, *unpublished data*) suggests that, in some FPA-r mutants, resistance to FPA is associated with changes in ribosome function. The evidence for this view is largely circumstantial, and the situation is currently being investigated in more detail. Of interest, however, is the fact that, if some FPA-r mutants are made streptomycin-resistant, there is suppression of the *fpa* mutation to give an FPA-s phenotype. The *fpa* and *str* loci involved are very closely linked (139). Other *fpa* loci are not linked to *str* and do not show this suppression. This raises the interesting question as to the relation of ribosome function and HCM, a relationship originally suggested by Lederberg (86). It is possible that some *fpa* mutations result in altered ribosome function, and that this altered function will result in errors in protein synthesis and hence changes in enzymes and substrates needed for HCM functions which are reflected by changes in Res and Mod phenotypes. FPA-r mutants which display such altered HCM properties also have much reduced growth rates on normal media (Dunn, *personal communication*).

It is not known how this range of genetic controls for HCM in *P. aeruginosa* is related to the actual mechanisms by which DNA specificity is imparted or recognized. For example, a number of genes have been shown to effect restriction, but it is not known whether there is only one restriction enzyme or a number of such enzymes, each with its specificity characteristics. It is clear that not only is the study of HCM valuable in its own right, but that the phenomenon has ramifications for a wide variety of cellular functions. Of particular importance are the relationship of HCM to bacterial conjugation and the concept of DNA specificity in terms of recombination function. These topics will be discussed below.

Other Types of Restriction

One of the features of HCM is, of course, the reduced ability of a bacteriophage to multiply in a particular bacterial strain. Other examples of such interference in phage multiplication occur in *Pseudomonas*, probably owing to mechanisms other than those associated with HCM. One such example has already been referred to above, where the presence of the F116 prophage prevents multiplication of phage B3.

In another case, the effects on phage multiplication appear to be due to a combination of bacterial genome and environmental factors (111). Multiplication (but not adsorption) of phage CB3 is prevented in certain strains of *P. aeruginosa* at incubation temperatures below 32 C. Prevention of phage multiplication enables the survival of the infected bacterium. By crosses between various strains of *P. aeruginosa*, segregation of bacterial genes affecting this characteristic can be shown. Several features, including the absence of multiplicity activation, provide evidence that failure of the phage to multiply is not due to breakdown of the incoming phage DNA by a restriction enzyme, and temperature-shift experiments suggest that an early event in the lytic cycle of phage CB3 is involved.

Strain-specific inhibition of phage multiplication has been described for *P. putida* phage pf1hd1 and the *P. putida* strains A3.12 and C1S (107). Phage pf1hd1, propagated on C1S, forms plaques with a very low efficiency on A3.12. Inhibition of the multiplication of phage pf1hd1 in A3.12 can be deduced not to occur by restriction enzymes which break down the incoming phage DNA, because some of the steps of phage multiplication can be detected in this bacterial strain. These include the synthesis of phage protein and phage DNA and also the lysis of the infected

bacterium, without, however, release of new mature phage. Mutants of pf1hd1 which have a high plating efficiency for *P. putida* A3.12 could readily be isolated. The results suggest that, in the wild state, this phage carries a nonsense mutation affecting a step leading to the formation of complete phage particles. As pf1hd1 plates normally on *P. putida* C1S, it can be suggested that this strain carries a suppressor gene, whereas the failure to plate on *P. putida* A3.12 is due to the absence of a suppressor mutation in the strain. Of 33 Biotype A strains of *P. putida* tested, 7 suppress the mutations of pf1hd1 and, of the strains tested, only *P. putida* A3.12 can differentiate between the normal and the mutant phage.

It can be concluded that in *Pseudomonas*, as in other bacteria, there are a number of restrictive mechanisms which may prevent phage propagated on one strain from multiplying in another. The action of these mechanisms is not only to limit phage infection but also to provide effective barriers to hybridization. Some of the phages which are subject to various types of restriction are known to be capable of transduction, and, in view of the demonstration (*see below*) that a *P. putida* phage may act as a fertility agent, these restrictive mechanisms may thus act as a control for the mechanisms by which DNA transfer can take place.

GENETIC RECOMBINATION

The list of bacteria with established recombination mechanisms continues to increase, particularly when some characteristic of the bacterium involved has the need for genetic analysis. *Pseudomonas* is one such case. Conjugation in *P. aeruginosa* was first studied at a time when it was desirable to extend the knowledge of this phenomenon to bacteria other than *E. coli* K-12. The continued study of genetic phenomena in this species, and more recently the discovery of genetic recombination in *P. putida*, has made available the benefits of this type of genetic analysis to this bacterial group.

Conjugation in P. aeruginosa

The first demonstration (57) of conjugation in *P. aeruginosa* used the same techniques which had proved successful in *E. coli* (84), namely plating mixtures of different auxotrophic strains onto a minimal medium which would not support the growth of either parent. In the first experiments, auxotrophs from four unrelated parental strains were crossed to each other to allow for the possibility that mating type differences could determine the occurrence of recombination. The results showed that, of these four strains, three were donors and one was a recipient strain. Prototrophic recombinants were formed in the successful matings at frequencies varying from 10^{-8} to 10^{-5} per parental cell. Two strains were selected for more detailed investigation. PAT2 is a genetic donor and possesses the mating factor FP. PAO1 is a recipient and is FP^-. Mating takes place in $FP^+ \times FP^-$ combinations; $FP^+ \times FP^+$ matings are fertile at very low frequencies (10^{-8}/parent cell or less; 58), and $FP^- \times FP^-$ crosses are completely sterile. PAT2 has not been shown to be able to transfer the sex factor FP to FP^- strains in the absence of chromosome transfer, in a manner analogous to the infectious transfer of F from F^+ to F^- strains in *E. coli*. However, some of the recombinants (up to 50%) of a PAT2 $FP^+ \times$ PAO1 FP^- cross become FP^+ and are found to be infectious for FP. These can transfer FP to FP^- strains without concomitant transfer of chromosome, and have the ability to convert to the FP^+ (or donor) state up to 80% of a female population with which they are in contact. The actual frequency of conversion depends upon the time that the FP^+ and FP^- cells are in contact and on the input ratio of FP^+ and FP^- cells. By this procedure, FP^+ derivatives of PAO1 FP^- strains can be readily prepared (65).

It thus appears that FP determines the occurrence of mating in *P. aeruginosa*, in a manner similar to the control of mating by F in *E. coli*. The availability of FP^+ and FP^- forms of PAO1 enables intrastrain crosses to be carried out, thus eliminating a source of error inherent in linkage data derived from PAT2 $\times$ PAO1 crosses, these strains having native differences in DNA specificity as described above. The effect of DNA specificity on conjugation and linkage will be discussed in a later section.

It was subsequently shown (92–94, 96, 97, 121, 129, 130) that $FP^- \times FP^+$ matings can be carried out by using an interrupted mating technique. This has not only made possible a better understanding of conjugation in *P. aeruginosa* but has also enabled a more valid comparison to be made with the more widely investigated *E. coli* conjugation system. Conjugation in *P. aeruginosa* will be discussed from a number of viewpoints.

Nature of the Sex Factor FP

Conjugation between strains of *P. aeruginosa* depends upon the sex factor FP being present in at least one of the parents. The kinetics of transfer of FP from FP^+ to FP^- strains appear to be somewhat different from that of F in *E. coli*.

Different male strains show a variation in the ease with which FP can be transferred. PAT2, a natural male strain, does not transfer sex factor to the female PAO1 at detectable frequencies. There could be a number of reasons for this, including the differences in DNA specificity between PAT2 and PAO1 and also the fact that PAO1 is aeruginocinogenic (*see below*), PAT2 being sensitive to the aeruginocin released from PAO1. However, use of a Res^- PAO1 and an aeruginocinogenic-resistant PAT2 strain does not promote FP transfer in this combination of strains, so it seems that PAT2 possesses some innate characteristic which prevents ready transfer of the sex factor to PAO1.

As described above, recombinants from PAT2 × PAO1 crosses, such as PTO629 (65, 129) or PTO13 (129), are much more infectious than most PAO1 FP^+ strains. Even after 2 hr of contact with an infectious PAO1 FP^+ strain, for example OT15 (derived from an FP^+ derivative of PAO1; reference 96), less than 10% of the recipients have acquired FP^+, these figures being much lower than would be found in a comparable situation with F^+ and F^- strains of *E. coli*.

It has not been possible to observe conjugation of *P. aeruginosa* cells under the microscope. Furthermore, there is no evidence that the presence of FP induces the formation of a structure analogous to the sex pilus of the *Enterobacteriaceae*. Electron microscopic observations (Holmes, *personal communication*; Veitch, *personal communication*) of a range of donor and recipient strains of *P. aeruginosa* have revealed no difference in the surface anatomy of these two types.

It is likely that this absence of structures involved in conjugation and the kinetics of FP infectivity may be due to a situation analogous to that described by Meynell, Meynell, and Datta (104) for some strains of *E. coli* possessing R factors. Such R factors can act as sex factors but usually at a much lower general efficiency than F. It is believed that, with such R factors, repression of the sex factor activity is very common, in contrast to the *E. coli* sex factor F whose functions are evidently completely derepressed. The fact that sex activity of strains which possess R factors occurs at all is due to derepression of sex factor function which permits formation of pili and chromosomal mobilization in a small fraction of the cells. Derepressed mutants of R-containing strains can be readily isolated, and these are found to possess sex pili in a majority of cells and to conjugate at a higher frequency more characteristic of F^+ strains.

Efforts to demonstrate a similar situation in *P. aeruginosa* by the isolation of derepressed mutants have so far proved unsuccessful. In fact, efforts to obtain fertility variants of any kind have had only limited success. If the FP factor is comparable to the *E. coli* F factor, then it should prove possible to isolate forms comparable to Hfr strains. Despite efforts over some years in several laboratories, no true Hfr form has yet been isolated. However, if FP is usually repressed, the isolation of such an Hfr form by the usual methods would obviously become much more difficult. The absence of sex-specific pili may also explain the failure to isolate sex-specific RNA phages (Holloway, *unpublished data*; Holmes, *unpublished data*).

In addition, FP is far more resistant to the action of acridines and metal salts than is the *E. coli* sex factor, and it has not been possible to "cure" *P. aeruginosa* strains of FP by such agents, under conditions found to be successful in other organisms. However, by treatment with the acridine half mustard ICR191, a mutant of an FP^+ strain has been isolated which has apparently lost the sex factor and which behaves as an FP^- strain (130). This acts as a normal recipient in crosses with FP^+ strains and can be reinfected with FP^+ to produce a normal donor strain.

It appears that when genetic material is being transferred from FP^+ to FP^- strains, the sex factor is far more susceptible to the action of acridines. This is one conclusion that can be reached from the effects of acridines on the transfer of chromosome. It is well established that acridines such as acriflavine or proflavine have little bacteriostatic or bacteriocidal effect on *P. aeruginosa* (1). This is not due to failure of the acridines to penetrate the bacterium as shown by a variety of effects of acridines on conjugation. At concentrations of 15 μg/ml, acriflavine strongly inhibits the transfer of chromosome during interrupted matings and also the infectious transfer of FP to FP^- strains from FP^+ strains (130). These effects are not caused by action of the acridine on cell pairing, nor is there any effect on recombination in the female parent. In view of the strong similarity between these results and those of Cuzin and Jacob with *E. coli* (30), it is postulated that the action of acridines is on the FP factor and that, in cells which can transfer FP as well as chromosome, the sex factor is in an acridine-sensitive condition not found in the majority of vegetative cells. It is likely that this sensitivity is associated with derepression of the sex factor. As the proportion of such derepressed cells will always be in the minority, this would explain the lack of apparent curing of the sex factor FP by acridine, as such curing cannot be readily detected at frequencies less than 0.1%.

Other attempts have been made to alter the properties of FP. Treatment of the male strain PAT2 with the acridine half mustard ICR191 has produced a variant with increased donor ability, so that it produces recombinants with the normal strain FP+ PAT2 at 1,000 times the normal frequency. It will also produce recombinents with FP+ and FP− derivatives of PAO1 at greater than normal frequency. If this mutant strain is crossed to PAO1 FP−, recombinants which become FP+ do not show this higher donor ability when crossed to other PAO1 FP− strains. From these and other data it is concluded that a chromosomal locus rather than the sex factor has been mutated to produce these fertility effects (130).

The inheritance of sex factor has been studied in interrupted matings (96, 129). The entry of FP into the female is very rapid and, in fact, FP seems to enter first, with little correlation between the transfer of FP and the transfer of chromosome. Up to 50% of recombinants may become FP+ and in some crosses there is a suggestion that the frequency of transfer of FP may vary with the particular selective markers used. This pattern of inheritance of FP is not consistent with the simple attachment of FP to the chromosome, resulting in terminal transfer of some of the FP genetic material. In such a case, which is analogous to the Hfr form of *E. coli*, we would expect almost all the recombinants to be FP−, which is not the observed result. A number of other possibilities may be suggested. Perhaps there are a number of copies of the sex factor present in the cell, and, whereas one of the unattached copies transfers early in conjugation another is associated with the chromosome and is responsible for its mobilization. Alternatively, sex factor and chromosome may be transferred independently, FP not being concerned with the transfer of chromosome, but only with the establishment of mating pairs.

Transfer of Chromosome

The transfer of chromosome is detected by the formation of recombinants. In earlier experiments, washed saline suspensions of each parent were mixed and plated onto selective media. Plate matings, however, whether in PAT2 FP+ × PAO1 FP− or PAO1 FP+ × PAO1 FP− crosses, showed that selection for different markers resulted in differences in recombination frequency up to 100-fold. Initial attempts to do interrupted matings with PAT2 × PAO1 crosses were not successful, probably due to the killing of the PAT2 parent by the aeruginocin produced by PAO1. Moreover, the differences in DNA specificity demonstrated by HCM between strains PAO1 and PAT2 created difficulties (119). For this reason almost all the linkage and conjugation studies have been done with PAO1 FP+ × PAO1 FP− crosses, one parent being made male by infection with FP as described above (65).

Preliminary linkage data have been obtained from plate matings (28, 64, 129), and one feature of these results is that there is a considerable variation in the recovery of different FP+ markers. This fact, combined with other linkage data, can be taken to mean that not all the genome of the male parent has an equal opportunity of being recovered in a recombinant. Furthermore, by making each parent resistant in turn to the virulent phage E79 and treating a mating mixture with this phage, it was shown that the transfer of chromosome was unidirectional, from the donor or FP+ parent to the recipient FP− parent (64).

These characteristics suggest a pattern of conjugation behavior superfically similar to that of *E. coli*. Knowing the history of genetic analysis in that organism, it became obvious that more meaningful linkage data and a better understanding of how the chromosome is transferred in *P. aeruginosa* would result from interrupted matings in broth rather than from plate matings. Loutit and Marinus (93) described a technique of interrupted mating based on that in use with *E. coli* studies, and demonstrated that polarized chromosome transfer takes place in *P. aeruginosa*. A number of studies were then made by using such an approach (92–94, 96, 97, 121, 129). The techniques used are based on those devised for *E. coli* (32, 141, 142). In general, the results of these independent investigations are the same, and, where differences do occur, they probably result from the use of different strains and differences in technique. For example, the use of nitrate broth increases recombination frequency about sixfold (97).

The important result emerging from these various experiments is that, by studying the times and rate of entry of different markers, there is found a gradient of entry times for different markers varying from less than 2 to nearly 60 min. In addition, there is a differential recovery of different markers. Loutit (92) showed a 50-fold difference in the frequency of recovery of a range of different auxotrophic markers. In general, early markers show a high recovery frequency, and late markers have a low recovery value, but some early markers have low recovery frequencies also.

Interrupted matings have been carried out with a variety of male strains marked with different auxotrophic markers and derived in different ways, but all possessing the FP factor from PAT2.

In all these crosses, the time of entry of a particular marker has remained constant and does not vary with different conditions or different parents.

It has already been pointed out above that there is prima facie evidence for the view that FP is concerned with the transfer of the chromosome. A detailed examination of the results of interrupted matings, in particular the wide range of entry times of different markers, suggests that $FP^- \times FP^+$ crosses in *P. aeruginosa* are more typical of an Hfr $\times$ F^- cross in *E. coli* than of an $F^+ \times F^-$ cross in the latter organism. An analysis has been made of the relative recovery of different markers entering at different times. In some cases, e.g., *met-28* and *ilv-2* which enter close together (28 and 30 min, respectively), their linkage on the chromosome is confirmed by the cotransduction of these markers by the phage F116 (129). In some cases selection for late-entering markers is accompanied by concomitant entry and recovery of earlier markers, as would be expected if there is a specific transfer origin on the chromosome. However, with other late entry markers, there is only a very small recovery of earlier markers, suggesting that in these cases the transfer origin is not the same as in the previous case where there was a high recovery of early markers. The data from Loutit and his colleagues (92, 94, 97) and Stanisich and Holloway (129) are consistent with the view that if FP does promote the transfer of chromosome by producing a transfer origin, then there must be more than one but perhaps only two or three such origins, and the evidence is not consistent with there being a multiplicity of transfer origins as is known to occur in *E. coli* K-12 (134).

Furthermore, the analysis of linkage data (92, 94, 129) can be interpreted as showing two or three linkage groups; these may correspond to segments of only one chromosome adjacent to a limited number of transfer origins. Linkage maps involving a number of markers have been produced. It is not clear yet, however, whether *P. aeruginosa* has only one chromosome or more than one chromosome, or indeed whether the chromosome is circular or linear. Loutit (92) and Loutit and Marinus (94) have mapped two groups of markers—those showing early entry (up to about 28 min) and those showing later entry, and, in all, 23 markers were located over a total span of 57 min; but, with the recombination frequencies available, reliable data are possible only for markers entering up to about 40 min after the parents are mixed.

If FP can produce a transfer origin at only a few sites, it is obviously more difficult to establish the precise nature and configuration of the chromosome than if there is a variety of sites. Perhaps FP factors from strains other than PAT2 may provide a different spectrum of transfer origins. To obtain a complete picture of the *Pseudomonas* genome, data are also required on the amount of DNA per nucleus and the number of nuclei per cell.

Although much remains to be discovered about the chromosome map of *P. aeruginosa*, it is worthwhile to compare what is known of the gene arrangement with that in other bacteria. Certainly there does not appear to be any overall similarity with that of *E. coli*, *S. typhimurium*, or *B. subtilis*, as can be seen by taking loci common to the three species such as streptomycin resistance, or individual loci in one or more biosynthetic pathways, and comparing the neighboring genes for each organism.

In contrast to the linkage results obtained by conjugation, analysis by transduction has revealed differences in the linkage relationships of genes controlling related functions.

HCM and Conjugation

From the study of HCM in *P. aeruginosa* phages, it can be concluded that the two interfertile strains PAO1 and PAT2 show different DNA specificities (*see* section above on HCM). Such differences in DNA specificity can affect conjugation, and in fact the degradation of incoming DNA by nucleases can provide an effective barrier to recombination, the genes controlling restriction in the recipient acting in effect to produce a recombination-deficient phenotype. It is known, furthermore, that such genetic differences in DNA specificity can affect linkage data (117). In the original plate-mating crosses of *P. aeruginosa* (PAT2 $\times$ PAO1; 57), one of the features of the results was the great variation in recombinant numbers isolated when different auxotrophs were mated, the differences observed being much more than in the PAO1 $FP^+ \times$ PAO1 FP^- crosses already described. Much of this variation arises not from any feature of the mechanism of chromosome transfer but from restriction of the chromosomal DNA from the PAT2 donor by the PAO1 recipient. When the PAO1 recipient is made restriction-deficient by growing it at 43 C (119) or mutating it to *res*$^-$ (120), recovery of some markers from PAT2 increases by as much as 500-fold, and linkage values are concomitantly altered.

These effects can also be produced in PAO1 $FP^+ \times$ PAO1 FP^- crosses when an FP^+ male strain grown at 43 C is mated to a 37 C-grown FP^- (in which restriction functions normally). Growth at 43 C leads to the synthesis of DNA

with different specificity from the normal 37 C type, and such a cross results in a much lower recombination frequency and altered linkage values, owing to the restriction by the FP$^-$ of the FP$^+$ DNA. This effect can be overcome by growing the FP$^-$ parent at 43 C, to make it restriction-deficient, so that it no longer degrades the male chromosome. It must be stressed that actual mating in all these experiments takes palce at 37 C. This can be achieved because these altered restriction and modification characteristics of the parent strains persist for up to 40 generations after the 43 C-grown cultures are returned to 37 (119) C.

When interrupted matings are carried out between parental strains in which the female parent restricts the DNA of the male parent, reduction of the recombination frequency depends upon the time of entry of the marker, early markers being more affected than late markers (121). These results support the notion of an oriented entry of markers, the amount of DNA required for entry of the later markers being more than that for earlier markers, and the additional DNA in the case of the later markers presumably causing saturation of the restriction enzymes in the female. Such effects can be eliminated by making the female parent restriction-deficient, (11, 119, 121).

It is generally accepted that the mechanism of restriction applies equally well to all types of DNA—phage, bacterial, and plasmid. One result with *P. aeruginosa* suggests that the sex factor FP may not be subject to restriction. It has been shown (67) that some recombinants from PAT2 × PAO1 crosses have DNA specificities which differ from either parent. At least some of these recombinants, as well as others with a DNA specificity like that of the PAO1 parent, can transfer the FP sex factor to PAO1 FP$^-$ strains at high efficiency (65, 129). The behavior of two such strains, PTO629 and PTO13, can be compared. Both transfer sex factor to FP$^-$ strains with equal facility. However, PTO629 has a different DNA specificity from PAO1, as shown by its ability when acting as a donor to transfer an early marker, such as *his-67* (7 min) to Res$^-$ but not to Res$^+$ females (121). The efficiency of sex factor transfer from PTO629 to a Res$^-$ PAO1 FP$^-$ is only slightly better than if a Res$^+$ PAO1 FP$^-$ recipient is used. By contrast, PTO13 transfers *his-67* to Res$^-$ and Res$^+$ females at equal frequency and has the same modification properties as PAO1 (Dunn, *personal communication*). If transfer of sex factor only involves the transfer of a small piece of DNA (say 1 to 2% of the total chromosome), then it is logical to expect that the sex factor from PTO629 would be restricted by recipients such as Res$^+$ PAO1 FP$^-$ in the same way as early markers such as *his-67*. It can be concluded that either the sex factor FP is immune to restriction mechanisms, unlike F in *E. coli*, or it does not acquire the host specificity of the bacterial strain in which it replicates.

Several *Pseudomonas* bacteriophages, including F116 and E79, are not subject to HCM (118). The reason for this is not known, but from the above observations it is likely that such an immunity to restriction and modification may extend to FP. It is clear that a sex factor which is not subject to restriction must have a selective advantage over one which may be destroyed by this mechanism.

These results stress the importance of DNA specificity in the detection and analysis of bacterial recombinants in intrastrain crosses and indicate how such specificity differences act to preserve the genetic integrity of strains. As such, the occurrence of DNA specificity must have been of considerable evolutionary significance in the general development of bacterial recombination mechanisms. Furthermore, restriction can provide different degrees of selection for markers on different regions of the chromosome. From current studies on conjugation, it is likely that the number of transfer origins on the *P. aeruginosa* chromosome is limited to perhaps two or three. Furthermore, it is known that, in matings between strains which have differences in DNA specificity, the recovery of early markers (but not late markers) is appreciably reduced. This means that the spread of particular markers from one strain to another may well depend upon the relative position of such markers in relation to the transfer origin. Such a situation would not be particularly important in organisms such as *E. coli* or *S. typhimurium*, where there are many alternate transfer origins, or in *B. subtilus* or *Haemophilus influenzae*, where conjugation is probably not an important mechanism of gene transfer, but it could have importance for *P. aeruginosa*. The distribution of different types of markers in the "early" and "late" regions of the chromosome may provide information of considerable evolutionary and biochemical interest.

In summary, the process of conjugation in *P. aeruginosa* is essentially similar to that occurring in *E. coli*. Most of the differences lie in what can be supposed to be a repressed or inactive state of the sex factor FP, compared with the active state of F in *E. coli*, and in the fewer chromosomal transfer origins which can be initiated by FP. This means that different regions of the *P. aeruginosa* chromosome have varying abilities to be transferred. It is not known whether the lower frequency of transfer of late markers is due to some

characteristics of the process of chromosome mobilization or whether mating pairs are too fragile to remain in conjugation for the time necessary to transfer these later genes.

Conjugation in P. echinoides

Heumann and Marx have studied genetic recombination in *P. echinoides*, a species characterized by star formation (54). This occurs through the close association of the fimbriae of cells of this species, resulting in an array in which they are joined at one end to form a starlike cluster. By using auxotrophic and pigment formation markers, genetic recombination was demonstrated and a preliminary chromosome map was prepared. In contrast to *P. aeruginosa*, this species does not show any sexual differentiation (53). The precise relationship, if any, between star formation and genetic exchange in this organism remains to be determined.

Transformation

Transformation in *Pseudomonas* was first reported by Lambina and Mikhailova (82) for *P. fluorescens*. Subsequently, Khan and Sen (80) reported both intraspecific and interspecific transformation in the genus. In general, there seems to be no difference in the details of this system from the more widely studied pneumococcus, *Bacillus*, or *Haemophilus* systems. Competent bacterial cells are obtained by the technique developed for *Haemophilus*. Transformation takes place at 30 C, and up to 5% of the cells treated with transforming DNA give rise to recombinants.

Transformation was shown to occur between different species of *Pseudomonas*, including *P. aeruginosa*, *P. fluorescens*, and several unidentified strains, and a range of markers can be transformed, including gelatin liquifaction and auxotrophy. Interspecific transformations can be carried out between species isolated from a wide variety of habitats; the species involved include plant pathogens, human pathogens, and soil inhabitants. Extension of the technique to biochemically known species of *Pseudomonas* or for the study of such phenomena as HCM in *P. aeruginosa* will provide a powerful investigational tool.

Transduction in P. aeruginosa

Not only is lysogeny extremely common in *P. aeruginosa*, but it appears that many of these temperate phages are capable of transduction. Five have been described in detail: B110 (66), B3 and F116 (70, 72), B (89–91), and G101 (69). All are general transducing phages with transducing frequencies up to 10^{-5} per recipient bacterium. In every case tested, the transducing particle has been found to be defective in phage function (105). Specialized transduction similar to that effected by λ and $\phi 80$ in *E. coli* has not been reported for *Pseudomonas*.

TABLE 4. *Cotransduction frequencies of linked markers with different phages used in P. aeruginosa*

Transducing phage	Marker combinations (% cotransduction)	
	str-2–trp-6	*met-28–ilv-2*
B3	0	1
F116	5	11
G101	4	34

In general, the properties of the *Pseudomonas* transducing phages are the same as those of the generalized transducing phages in *E. coli* or *S. typhimurium*. Investigations of the physical and structural properties of two of these transducing phages, B3 and F116 (31, 126), have shown that the DNA content of B3 is 20×10^6 to 25×10^6 daltons and that for F116 it is 40×10^6 to 50×10^6 daltons. The larger size of F116 DNA is reflected in the more frequent occurrence of cotransduction of markers mediated by this phage.

In Table 4, the various cotransduction frequencies for three transducing phages are given. The marker combinations *str-2 trp-6* and *met-28 ilv-2* show close linkage in conjugation; F116 shows more frequent cotransduction than B3. Nothing is known yet about the molecular size of G101 DNA, but this phage displays cotransduction of a number of marker combinations, so that it probably has a DNA content similar to that of F116, if not greater.

Density gradient studies (Lee, *personal communication*) with F116 have shown that the density of transducing particles is almost the same as that for normal active phage particles, suggesting that their DNA content is about the same. No accurate attempts have been made to measure the amount of DNA per cell of *P. aeruginosa* but, by comparison with *P. putida* and *E. coli*, it is reasonable to suggest a figure of 2×10^9 to 3×10^9 daltons. This means that F116 can transduce a maximum of about 1 to 2% of the genome of *P. aeruginosa*. Apart from a study of the effects of radiation on transduction in *P. aeruginosa* (72), the emphasis on most transduction studies has been on linkage analysis. This work is described below.

Transduction in P. putida

The detection of transduction in a species of *Pseudomonas* other than *P. aeruginosa* was first

made by Gunsalus and his colleagues (22) with the isolation of the transducing phage pf16 for *P. putida*. Subsequently another transducing phage, PP1, acting on different strains of *P. putida*, was independently isolated (68).

Pf16 has been characterized in some detail (108). It is not capable of establishing lysogeny, and, in view of recent work by Chakrabarty and Gunsalus (20), it is probably a nonlysogenizing form of a temperate phage. When used for transduction, optimal recombination frequencies are obtained at low multiplicities of infection (ca. 0.5) and with phage preparations that have been irradiated with UV. Under these conditions, transduction frequencies of 10^{-6} per recipient cell can be achieved. Phage pf16 contains about 10^8 daltons of double-stranded DNA and, hence, as the DNA content per cell of *P. putida* has been calculated at 2×10^9 to 3×10^9 daltons, it appears that transducing particles of phage pf16 could carry up to 5% of the *P. putida* genome. This view is supported by the extent to which cotransduction of markers is observed with this phage.

The somewhat unusual nature of phage pf16 has been demonstrated in interstrain transductions by using the host range mutant pf16h2 and strains PRS1 and PUG2 of *P. putida* (20). In these transductions, selection is made for the ability to use mandelate as sole carbon source. Intrastrain transductions with PRS1 as donor and acceptor give recombinants which are phage sensitive. However, interstrain transductions with PUG2 as recipient and PRS1 as donor produce immune transductants, which are unstable for selected markers and for immunity. The immune transductants behave as defective lysogens, and, on superinfection with plaque-forming particles, yield lysates which have a high frequency of transduction. In some cases phage-sensitive transductants of PUG2 behave in the same way, suggesting that these transductants have acquired a self-replicating, extrachromosomal genetic element containing both phage and bacterial DNA; the element has been called pf*dm*, analogous to λ*dg* or Pl*dl* in *E. coli*. It is suggested that the relative proportions of phage and bacterial DNA can vary—this feature being supported by the range of properties of different transductants and by data obtained by buoyant density centrifugation of the various types of transducing phage preparations. The phage-sensitive transductants contain a satellite band of DNA, suggesting that the transduced fragment is not integrated into the chromosome and that pf*dm* particles can replicate autonomously.

Defective Transducing Phage as a Fertility Factor in P. putida

In a further study (21) of the properties of the *P. putida* transducing phage, pf16, conjugation was found to occur when strains that had been transduced for the mandelate character, and which harbored pf*dm* particles, were used as donors in mating experiments with recipients that did not possess pf*dm*. It was possible to eliminate transduction or transformation as the cause of the gene transfer: it thus appears that pf*dm* particles can act to promote the transfer of chromosome. In all cases the transfer of chromosome is accompanied by the transfer of pf*dm*, so the phenomenon shows some similarities to F′ transfer in *E. coli*.

GENE ARRANGEMENT IN PSEUDOMONAS

The transducing phages of both *P. aeruginosa* and *P. putida* have been used to study the linkage arrangement of genes, particularly with respect to genes related to a given pathway. In any linkage studies in bacteria, it is important to recognize any differences between different organisms; in this respect *Pseudomonas* does show a different pattern of gene distribution from that found in *E. coli* and *S. typhimurium*. In both these latter organisms it is found that genes controlling sequential steps of many biosynthetic or catabolic pathways tend to be arranged contiguously on the chromosome (34, 123, 124). For example, in both *E. coli* and *S. typhimurium* the genes controlling the synthesis of histidine are clustered together, as are those for leucine and tryptophan biosynthesis. This gene arrangement has been found to have significance for control of enzyme biosynthesis (76). Not all groups of related genes are arranged in this way, and this type of arrangement is probably less common than was originally supposed (34, 134). The evidence to date in *Pseudomonas* indicates that, for the biosynthetic pathways, the related markers of any one pathway are not arranged contiguously. Instead, such related genes are found to be arranged in clusters consisting of only two or three genes, and in several cases genes within these clusters appear to be contiguous. The remainder of the genes in each pathway are scattered individually and not closely linked to each other.

In *P. aeruginosa*, Holloway et al. (71) and Fargie and Holloway (38) surveyed a number of pathways by means of transduction analysis for the linkage of related genes. It was shown that there was no contiguous arrangement of all the structural genes for any of the following path-

ways: tryptophan, leucine, histidine, isoleucine-valine, pyrimidine, purine, methionine, proline, arginine, and cysteine. Subsequently, a number of these pathways have been examined in more detail (18, 75, 101, 103, 116).

Mee and Lee (103), using phage F116, carried out a transduction analysis of 107 histidine-requiring mutants derived from PAO1. These mutants could be classified in five separate groups, each group being unlinked to any other group. At least two of these groups probably consist of more than one gene, and it is clear that in *P. aeruginosa* the structural genes of the histidine pathway are not all contiguous, as occurs in *S. typhimurium* (3) or *Staphylococcus aureus* (81), but are arranged such that some genes are clustered and others are scattered. In this respect *P. aeruginosa* is more like *Neurospora* and *Saccharomyces*, where there are seven linkage groups for genes controlling histidine biosynthesis.

A similar type of genetic analysis has been undertaken with the isoleucine-valine pathway (101, 116). Ninety-three isoleucine- and valine-requiring mutants derived from *P. aeruginosa* strains PAO1 and 78 were analyzed by use of the transducing phages F116 (for PAO1) and B (for strain 78). These mutants could be placed into three functional groups: *ilvB* lacking acetohydroxy acid synthetase, *ilvC* lacking reductisomerase, and *ilvD* lacking dihydroxy acid dehydratase. The loci *ilvB* and *ilvC* are possibly contiguous, but *ilvD* is not situated close to either *ilvB* or *ilvC*. In this respect, the distribution of *ilv* genes is unlike that in either *E. coli* or *S. typhimurium*, in which all the *ilv* genes are contiguous (123, 134), but is similar to that in *B. subtilis* where some *ilv* loci are clustered and some are not.

In *P. putida*, a similar pattern of distribution is found for the genes affecting tryptophan biosynthesis (22, 45). By means of the transducing phage pf16, three gene clusters were identified. One contains the genes *trpA* (anthranilate synthetase), *trpB* (phosphoribosyl transferase), and *trpD* (indoleglycerol phosphate synthetase). The second cluster consists of *trpE* (tryptophan synthetase A protein) and *trpF* (tryptophan synthetase B protein), whereas the third consists of only one gene, *trpC* (phosphoribosyl anthranilate isomerase). All three groups are unlinked to each other. A less detailed transduction analysis of *P. aeruginosa* with phage F116 showed that there were three groups of tryptophan loci; the gene distribution for this pathway seems to be the same for both *P. aeruginosa* and *P. putida* (38). As pointed out earlier, genes controlling HCM in *P. aeruginosa* are unclustered, unlike the situation in *E. coli*.

Studies have been made on the linkage of genes that specify inducible enzymes functional in the degradation of various substrates in both *P. putida* and *P. aeruginosa*. The utilization of one such substrate, mandelic acid, has been studied in some detail (50–52). The details of the pathway and the enzymes concerned are shown in Fig. 1.

D (-) MANDELATE
mandelate racemase
L (+) MANDELATE
L (+) mandelate dehydrogenase
BENZOYLFORMATE
benzoylformate decarboxylase
BENZALDEHYDE
NAD + NADP linked dehydrogenases
BENZOATE
ben
benzoate oxidase
CATECHOL
catA
catechol oxygenase
cis, cis - MUCONATE
catB
cis, cis - muconate lactonizing enzyme
MUCONOLACTONE
catC
muconolactone isomerase
ANTHRANILATE
ant
anthranilate oxidase
p-HYDROXYBENZOATE
pobA
p-hydroxybenzoate hydroxylase
PROTOCATECHUATE
pcaA
protocatechuate oxygenase
β-CARBOXY cis,cis-MUCONATE
pcaB
β-carboxy muconate lactonizing enzyme
γ-CARBOXY MUCONOLACTONE
pcaC
γ-carboxy muconolactone decarboxylase
β-KETOADIPATE ENOL-LACTONE
pcaD
β-ketoadipate enol-lactone hydrolase
β-KETOADIPATE
pcaE
β-ketoadipate Co-A transferase
β-KETOADIPYL -CoA

FIG. 1. *Mandelate-benzoate pathway in P. putida.*

P. putida PSR1 is able to oxidize mandelate, benzoate, and *p*-hydroxybenzoate. Another strain, *P. putida* PUG2, lacks the *p*-carboxy-muconate-lactonizing enzyme (CMLE) of the *p*-hydroxy-benzoate pathway and thus will not grow on *p*-hydroxybenzoate. Strain PUG2 also fails to grow on D-mandelate, L-mandelate, or benzoylformate but will grow on benzoate.

With PSR1 as the donor, and a host range mutant of the transducing phage pf16, it was shown that the genes for the mandelate pathway are not linked to that for CMLE in the *p*-hydroxy-benzoate pathway. However, cotransduction of four genes controlling mandelate function was shown (22).

Preliminary evidence in *P. putida* suggests that genes controlling two of the six steps in the degradation of D-camphor are closely linked. These genes showed cotransduction with each other and also with a marker for resistance to FPA.

Kemp and Hegeman (79) showed in *P. aeruginosa* that three loci governing enzymes of the pathway of β-ketoadipate utilization, *pcaB*, *pcaD*, and *pcaE* (*see* Fig. 1), are closely linked, as indicated by transduction analysis with phage F116; in fact, these loci are probably contiguous. No mutants were obtained for *pcaC* and there was no linkage of *pcaA* with the other markers of the protocatechuate branch. The markers of the catechol branch, *ant*, *catA*, *catB*, *catC*, *ben* showed linkage to each other but not to the protocatechuate group. The locus *pobA* was found to be linked, although not closely, to the *pcaB*, *pcaD*, *pcaE* group.

It is interesting to note that, although comparative mapping of *P. aeruginosa* and *P. putida* is in its early stages, a number of similarities have been revealed. The arrangement of a *trp* gene cluster in proximity to streptomycin-resistance and FPA-resistance genes is a feature of both species, as is the linkage arrangement of genes for the tryptophan, methionine, adenine, and leucine pathways. There is also a suggestion of similar distribution of genes for the *p*-hydroxybenzoate degradative pathway in *P. putida* and *P. aeruginosa* (Wheelis, *unpublished data*).

It can therefore be concluded that the pattern of gene arrangement found in *Pseudomonas* is different from that of the enterobacteria. It is difficult to assess the significance of this difference in overall terms, but in *E. coli* the localized arrangement of some genes is important for the control of enzyme biosynthesis. A number of investigations of enzyme control mechanisms in *Pseudomonas* have now been made to reveal patterns of such mechanisms different from those of other bacteria.

BIOCHEMICAL GENETICS OF PSEUDOMONAS

It is not the intention of this review to carry out a general survey of the metabolic, biosynthetic, and biochemical behavior of *Pseudomonas*, as this has already been done in a number of other reviews (27, 106, 128). However, because much of the current genetic interest in *Pseudomonas* arises from the biochemical properties of this genus, it is proper to discuss the contribution that genetic studies have made to this area and to indicate useful avenues of study.

There are two questions that can be asked. First, does the broad metabolic versatility of *Pseudomonas* allow for any general differences from, say, the *Enterobacteriaceae*, in the control of anabolism and catabolism? Second, does the relatively unclustered arrangement of related genes have any significance for control mechanisms generally in this genus?

In view of the importance of gene distribution in relation to gene control in *E. coli* (76), the observed distributions of genes for biosynthetic and other pathways reported above suggests that the pattern of control of such pathways may be different in *Pseudomonas*. As mentioned above, in *P. aeruginosa*, complete clustering of the structural genes of any of the biosynthetic pathways examined does not occur. Instead, the genes are arranged either as unlinked clusters of two or three genes or singly, but without any general contiguous arrangement. It is yet too early to come to any general conclusions concerning the genetic basis of enzyme control in *Pseudomonas* because so few pathways have been investigated and dominance studies are not possible at present. However, the data support the view that there is a functional significance in the observed arrangement of genes in this organism. It is convenient to examine in turn the data of the various biosynthetic and degradative pathways which have been studied.

Pyrimidine Biosynthesis in P. aeruginosa

In *E. coli* the distribution of genes in the pyrimidine pathway is such that coordinate repression has been shown for enzymes governed by both linked and unlinked genes (7). Isaac and Holloway (75) isolated 27 mutants of *P. aeruginosa* PAO1 requiring uracil for growth and identified them for their enzyme lesions. The genes controlling the four loci involved were shown to be unlinked to each other. In contrast to the situation in *E. coli* (7) or *Serratia marcescens* (49), no changes occurred in the levels of pyrimidine biosynthetic enzymes upon altering the concentration of uracil in the growth medium

or by growth in a complete broth. This lack of repression is also accompanied by a lack of derepression when mutants are grown in growth-limiting amounts of pyrimidine. With a bradytrophic mutant growing in minimal medium, where growth is limited by the uracil supply and where protein synthesis still occurs, no derepression of uracil biosynthetic enzymes was observed. A low level of feedback inhibition was demonstrated, but, despite the lack of repression and derepression, there is no evidence that feedback inhibition has any unusual significance for enzyme control. Thus, although the arrangement of genes in *P. aeruginosa* is essentially no different from that of other bacteria, with respect to the pyrimidine biosynthetic pathway, differences in the control of enzyme synthesis in this pathway have been established.

Isoleucine-Valine Biosynthesis in P. aeruginosa

Marinus and Loutit (102) have examined the pathway and control mechanisms of isoleucine-valine biosynthesis in *P. aeruginosa*. One feature found in *P. aeruginosa* and not *E. coli* is that one-step mutants can be isolated that require isoleucine and valine for growth and which are deficient in acetohydroxy acid synthetase (99). A study of the control patterns of the enzymes in this pathway showed that both acetohydroxy acid synthetase (AHS) and reductoisomerase (RI) exhibit about two- to threefold repression in the presence of isoleucine, valine, leucine, and pantothenic acid, whereas threonine dehydratase and dihydroxy acid dehydratase levels are invariate under varying conditions of end-product concentration. By using leaky revertant strains, up to 8- to 10-fold derepression of both AHS and RI was shown, but, under these conditions, the levels of other enzymes in the pathway were not altered. Moreover, when estimated under a variety of conditions, AHS and RI showed coordinate expression. It thus seems that *ilvB* (AHS) and *ilvC* (RI) constitute a genetic unit of coordinate expression and, in support of this view, mutants with pleiotropic effects have been found (100). For example, one mutant isolated is fully derepressed for AHS but contains no RI. This mutant maps outside the *ilvB* gene, on the side distal to the *ilvC* region.

These results suggest that *ilvB* and *ilvC* might comprise an operon, the other genes of this pathway being unlinked to this cluster. This situation is in contrast to the situation in *E. coli* and *S. typhimurium*, where all *ilv* enzymes can be coordinately repressed and the genes controlling them are all contiguous. Thus, in *P. aeruginosa* there exists an example of a relationship between the arrangement of genes and their control—the clustered genes show repression and the other scattered genes are constitutive. A similar arrangement has been reported by Gunsalus and his colleagues for the tryptophan pathway in *P. putida* (*see below*).

Tryptophan Biosynthesis in P. putida

A detailed biochemical study of the tryptophan pathway in *P. putida* has been made (22, 29, 45). In *E. coli*, the control mechanism for this pathway involves the coordinate repression of all the enzymes by tryptophan. In this case, all the genes are contiguous, in contrast to the *P. putida* system where there are three unlinked clusters of genes. The pathway of tryptophan biosynthesis in *P. putida* is the same as in *E. coli*, but the mechanisms of control are quite different. The level o tryptophan synthetase is controlled solely by induction, the inducer having no effect on earlier enzymes (29). The inducer is indoleglycerol-phosphate and no repression is exerted by tryptophan. On the other hand, by using a bradytrophic mutant, derepression of tryptophan synthetase can be demonstrated.

Three of the early enzymes—anthranilate synthetase, phosphoribosyl transferase, and indoleglycerol phosphate synthetase—are repressed by tryptophan but not in a coordinate manner, and, under conditions of limiting tryptophan, are derepressed 40-fold, 12-fold, and 11-fold, respectively. The genes specifying these three enzymes are closely linked, as shown by the transduction analysis. The remaining enzyme, phosphoribosylanthranilate isomerase, appears to be constitutive, and the gene controlling this enzyme (*trpC*) is unlinked to the other tryptophan loci.

Thus, the arrangement of genes and the occurrence of various mechanisms of control, in the tryptophan pathway in *P. putida* and in the isoleucine-valine and pyrimidine pathways in *P. aeruginosa*, suggest some common theme in this genus. Further evidence for this view comes from the study of a number of degradative pathways. As pointed out above, the ability of pseudomonads to use a variety of energy sources is an important feature of this group of organisms, and it is desirable to know how this feature of its metabolism is controlled.

β-Ketoadipate Pathway

This pathway is used for the degradation of aromatic substrates and has been extensively studied. Ornston (113), using *P. putida*, showed that there is a coordinate induction of the enzymes controlled by *pcaB*, *pcaC*, *pcaD*, but not *pcaE* (*see* Fig. 1), suggesting that the genes for this

group of enzymes constitute an operon. Kemp and Hegeman (79), in a combined genetic and biochemical investigation, have now shown that the various intermediates and the induction patterns of the β-ketoadipate pathway are the same in *P. aeruginosa* as in *P. putida*, and also that the product of *pcaE* is synthesized coordinately with those of *pcaB*, *pcaC*, and *pcaD* and that *pcaB*, *pcaD*, and *pcaE* are probably contiguous in *P. aeruginosa*, thus adding considerable support to Ornston's suggestion. This is also true for genes of the catechol pathway, in particular *catA*, *catB*, and *catC*, which also have a common inducer.

However, there is clustering of additional loci which do not share a common inducer. It is found that *pobA* is linked to, but not contiguous with, *pcaB*, *pcaD*, and *pcaE*, and Kemp and Hegeman have suggested a loose general clustering of the genes affecting aromatic metabolism in *P. aeruginosa* (79). However, the significance of this arrangement for control purposes is not clear. Further transduction studies (122) have shown that the genes for mandelate dehydrogenase and benzoyl formate decarboxylase, two sequential steps in the mandelate pathway, are not linked to each other, but each shows a low frequency of cotransduction with *ben* and the *catA*, *catB*, *catC* cluster. There is no linkage of mandelate genes to the genes of the *p*-hydroxybenzoate pathway.

Control of Amidase Formation in P. aeruginosa

P. aeruginosa produces an aliphatic amidase which hydroyzes acetamide and propionamide. This amidase is inducible by these and other nonsubstrate amides. Not only has this system permitted the study of such inducible enzymes (10, 15), but it has been possible to isolate regulator mutants of this system (16). By transduction studies with phage F116, it was shown that the amidase structural and regulatory genes were very closely linked. Those mutants which can be induced by formamide appear to have an altered inducer specificity, so that this amidase system in *P. aeruginosa* probably resembles the β-galatosidase operon in *E. coli* in the close proximity of structural and regulatory genes.

Discussion of Control Mechanisms

The work to date suggests that the mechanisms of enzyme control in *Pseudomonas* are not fundamentally different from those of other organisms but that the pattern of the mechanisms shows important differences. At present, it would be best to describe them as "variations on a theme," with the possibility that, to continue the musical analogy, a new theme may well be introduced in the next movement. For example, *Pseudomonas* can use amino acids, purines, or pyrimidines as sole carbon or nitrogen sources for growth, and it is possible that the control of such catabolic functions may in turn influence control of the biosynthesis of these substances.

These different patterns of control are perhaps to be expected when we compare the ecology of *Pseudomonas* with other bacteria. *E. coli*, as a normal inhabitant of the animal gut, comes from an environment in which large quantities of a rather constant food supply enter at intervals and must be disposed of in a limited time. *Pseudomonas* is an organism commonly found in soil or water, where the food supply would be irregular, varied, and often in low concentration. Obviously, a pattern of control mechanisms which enable a more diverse metabolic system to function efficiently will be needed for this varied environment, and this may well be quite different from that required for the optimal use of food supply by a bacterium in the animal gut.

At present, we would like to know whether the gene distribution in *Pseudomonas* is related to such a different pattern of enzyme control. It is my belief that there is a relationship, and, furthermore, that the *Pseudomonas* pattern of limited clustering of related genes more closely approximates the situation in other organisms (73)—in other words, the degree of clustering of genes found in enteric bacteria may be the exception rather than the rule. That gene distribution is only one of the factors which affects control mechanisms is seen by the recent observations of Baumberg et al. (6). These authors showed that the structural genes for acetylornithinase and argininosuccinase which are normally clustered in *E. coli* may become separated by means of a genetic rearrangement. Nevertheless, the control of these two genes, which involves a noncoordinate repression, is unchanged by the rearrangement, indicating that individual repressor recognition sites are involved.

One approach to the knowledge of genetic control in *Pseudomonas* would to be to isolate and study control mutants, analogous to the regulator and operator mutants isolated for certain enzyme systems in *E. coli*. Such mutants do occur: for example, the presumptive operator-constitutive mutants of the mandelate pathway isolated by Hegeman (52). An attempt was made to use analogue-resistant mutants for the isolation of control mutants in *P. aeruginosa* (139). It was argued that analogue-resistant mutants could have a modified control function and would be resistant, because overproduction of any particular amino acid, purine, or pyrimidine would

result from such alteration of biosynthetic control. Experiments showed, however, that *P. aeruginosa* was indifferent to a large number of such analogues (139) and was sensitive to only a few, including FPA. Mutants resistant to FPA could be readily isolated but, in fact, they were not found to overproduce phenylalanine. No evidence was found that they were control mutants, and it is thought that they represent ribosomal or transfer-RNA mutants (138; Dunn, *personal communication*).

An additional difficulty in studying the nature of genetic control in *Pseudomonas*, particularly in the characterization of mutants of the operator or regulator type, lies in the fact that no partial diploid structure is yet available in *Pseudomonas*. The characterization of such a structure must be one of the immediate aims of further research if the genetic basis of mechanisms governing the synthesis of protein in *Pseudomonas* is to be fully understood.

MUTANTS AFFECTING RECOMBINATION

The isolation of bacterial mutants which affect recombination function has enabled a more direct approach to the fundamental nature of recombination and has stimulated the search for such mutants in other organisms (25, 140).

A class of recombination mutants has been isolated in *P. aeruginosa* which appears to be somewhat different from those described for *E. coli* or *S. typhimurium*; the method used for their isolation was also different. Rec^- mutants of *P. aeruginosa* were sought as mutants deficient in the ability to undergo lysogeny, on the argument that a bacterial recombination function might be necessary for the integration of prophage. This hypothesis is now known to be incorrect for phages such as λ and $\phi 80$ in *E. coli*, where integration appears to be solely a function of the phage genome (124). Furthermore, the Rec^- mutants of *E. coli* can be lysogenized with the same frequency as that of the Rec^+ form (17). However, mutants of *E. coli* which have reduced ability to be lysogenized by phage P2 are Rec^- (125).

As described earlier in this review, mutants, which cannot be lysogenized at low multiplicities of infection may be readily isolated. Among these mutants are many which also exhibit a Rec^- phenotype in that they show reduced recovery of recombinants when used as recipients in both transduction and conjugation experiments. The extent of this reduction varies with different mutants, but the more extreme examples show less than one per cent of the normal recombination level (62). In considering this loss of recombination function by the mutants, the following mechanisms can be suggested (26): (i) mutation in the structural genes for recombination enzymes; (ii) changes in the control mechanisms for the synthesis of recombination enzymes; and (iii) changes in the substrate of recombination enzyme(s), such that normal activity cannot occur. This latter possibility would suggest that there is a specificity of DNA for recombination enzymes similar to that of DNA for restriction enzymes. DNA which has an altered specificity would thus not be a normal substrate for recombination enzymes, with resultant loss of recombination function.

Isolation and characterization of the enzymes involved is probably the only way in which these various possibilities can be distinguished. Certainly the Les^- mutants characterized show normal (wild type) restriction and modification function for phage infection, i.e., they are not Res^- Mod^+ or Res^- Mod^- (Holloway, *unpublished data*).

Recent evidence makes it more likely that changes in the substrate cause the altered recombination phenotype, rather than changes in the enzymes themselves.

Data to support this come from experiments in which Les^- Rec^- strains are used as donors rather than as recipients in conjugation or transduction. As bacterial recombination may be involved in either chromosome mobilization (for conjugation) or in the formation of transducing fragments of the bacterial chromosome (for transduction), a reduced recovery of recombinants may occur when Les^- Rec^- strains are used as donors. However, if normal DNA is being transferred, and with normal recombination enzymes functioning in the Rec^+ recipient, no aberrations of linkage would be expected among any recombinants that may be formed when Rec^- Les^- strains are used as donors.

However, the results with a variety of Les^- strains (Table 5) show that not only is the recombination frequency of such crosses usually much reduced but that linkage values of certain markers (*met-28* and *ilv-2* for transduction and *met-28* and *trp-6* for conjugation) show considerable deviation from that found with Les^+ donors.

One likely conclusion from these results is that the chromosomal material from a Les^- strain cannot show normal recombination function in a Les^+ strain. This suggests that there is a DNA specificity for recombination analogous to the specificity found in restriction and modification.

The lysogenic characteristics of these Les^- mutants may be consistent with this view. As already pointed out (136), these properties could be due to the different availability of repressor for lysogenic function in the mutant and wild-type

TABLE 5. *Recombination frequencies and linkage data for Les⁻ × Les⁺ recipient crosses in P. aeruginosa; transduction and conjugation (data from Holloway and Pemberton, in preparation)*

Donor[a]	Percent cotransduction *met-28, ilv-2*
PAO41 (*trp-4, les⁺*)	11.0
PAO803	0.0
PAO814	0.0
PAO815	4.1
PAO816	13.9
PAO810	3.6

FP⁺ donor[b]	Conjugation frequency[c] (% obtained with PAO381)	Cotransfer frequency *met⁺-28* and *trp⁺-6*
PAO381	100	34
PAO351	1	0
PAO353	5	24

[a] PAO803, 814, 815, 816, 817, 810 are various *les* derivatives of PAO41, all are *trp-4*. Transduction: recipient PAO8 (*met-28, ilv-2, les⁺* FP⁻). Transduction techniques used were those described by Fargie and Holloway (38).

[b] PAO381 is *leu-38*, FP⁺ *les⁺*; PAO351 and PAO353 are *les* derivatives of PAO381.

[c] Conjugation: recipient PAO286 (*met-28, trp-6,* FP⁻). Conjugation was carried out by plate mating using the technique described by Stanisich and Holloway (129).

cells. Such a difference could result from the presence of by-products of altered DNA metabolism reacting with the repressor (17). It has been shown (136) that, with one of these Les⁻ mutants, DNA synthesis is temperature sensitive, as is the recombination ability. Genetic evidence indicates that the same mutant site is involved in both these phenotypic effects.

OTHER FEATURES OF GENETIC INTEREST

Autoplaque Phenomenon

Many strains of *P. aeruginosa*, when cultivated on certain solid media, show the spontaneous formation of plaque like erosions on the surface of the bacterial growth. These have been described as "the beaten-copper effect" or "the metallic effect," but the term autoplaque, introduced by Berk (8, 9), seems best to describe the phenomenon. Autoplaques generally appear after about 12 hr of growth upon the surface of solidified, poorly buffered, rich media, and increase in size and number with further incubation when the culture has passed the logarithmic growth phase.

It is known that a range of treatments can alter the expression of the phenomenon by strains which show autoplaque production (AP⁺); these include nutrient levels, nitrogenous supplements antibiotics, sulfa drugs, and acridines. Mutation from AP⁺ to AP⁻ and the reverse have been described, and in my laboratory it has been observed that some AP⁻ strains become AP⁺ when they mutate from chloramphenicol sensitivity to resistance.

The plaques of some phages are remarkably similar to those characteristic of the autoplaque phenomenon, particularly in the occurrence of an irridescent sheen in the plaque. It has been suggested that the autoplaque phenomenon is of phage origin, but there are a number of reasons which make this unlikely. In the first place, if the effect resulted from a prophage it would have to involve a wholesale breakdown in immunity by the bacterial culture, or result from a rash of mutations by the prophage to overcome host immunity, both of which seem improbable. Secondly, the autoplaques do not appear during the logarithmic phase of growth of the bacteria but in early stationary phase, and increase in size and number during the continuation of stationary phase, a series of events quite distinct from the production of phage plaques. Finally, despite many attempts to do so, phage has never been demonstrated in the autoplaques. For the present, the genetic basis of autoplaque formation remains obscure.

Bacteriocins Produced by Pseudomonas

Aeruginocins (or pyocins) are the bacteriocins produced by *P. aeruginosa*. The occurrence of aeruginocinogenic strains is extremely frequent (47, 48) and has enabled techniques for typing strains of *P. aeruginosa* for epidemiological purposes (44, 59, 115).

The structure of a number of aeruginocins has recently been described and reviewed by Bradley (14). Some aeruginocin particles show a strong similarity to bacteriophage comqonents, and, in fact, *P. aeruginosa* PAOI and its derivatives release an aeruginocin of this type. Mitomycin treatment of *P. fluorescens* gives essentially the same type of particle. It is very likely that bacteriocins are common in *P. putida* strains, but the bacteriocins (putidacins?) from this species have not been studied in any detail. The physical and chemical properties of a number of aeruginocins have been studied (55, 77), but there have been few genetic studies on aeruginocin production. There are no reports of genetic transfers by

aeruginocinogenic factors, as occurs with colicinogenic factors in *E. coli* or *S. typhimurium*. Attempts to transfer aeruginocinogenic factors by infectious transfer were unsuccessful (47; Fargie and Holloway, *unpublished data*). This is an area of *Pseudomonas* genetics well worth further attention. If, as appears to be the case, bacteriocins are common in species of *Pseudomonas* like *P. putida* or *P. fluorescens*, then perhaps they represent a potential mechanism of genetic transfer for these species.

Drug Resistance

It is a common phenomenon in most general hospitals that the frequency of occurrence of infections due to *P. aeruginosa* is increasing. The reasons for the increase are not completely understood, but undoubtedly they bear some relationship to the widespread use of antibiotic therapy and the resistance of *P. aeruginosa* to most of the widely used antibiotics. It is not unusual for strains of this organism isolated from infections to be resistant to three or more of the following antibiotics: sulphadiazine, ampicillin, kanamycin, streptomycin, chloramphenicol, neomycin, or tetracycline. The only antibiotic to which *P. aeruginosa* is usually sensitive is polymyxin, although strains resistant to this agent are common. The reasons for this pattern of resistance to such a wide variety of drugs are not understood at the biochemical level. This, too, could be a fruitful avenue of future research, with both academic and practical implications.

The epidemiology of drug resistance in the *Enterobacteriaceae* and some of the gram-positive cocci is undergoing a remarkable change in character with the widespread occurrence of resistance transfer factors (RTF). RTF may be transferred to drug-sensitive strains by conjugation in much the same way and with much the same type of kinetics as F transfer in *E. coli*. Furthermore, RTF can act as sex factors in promoting conjugation and transfer of chromosome (132).

Given this drug-resistant nature of *P. aeruginosa*, it is important from a public health viewpoint to know whether RTF can either occur in this species or be transferred to it from the enterobacteria. Smith and Armour (127) showed that the drug resistance of *P. aeruginosa* could be transferred to sensitive *E. coli* cells when the strains were mated on membrane filters (Millipore Corp., Bedford, Mass.), such mating in broth being sterile. The efficiency of transfer was far lower than in similar types of transfer by *E. coli* strains possessing RTF. No attempts were made in this study to transfer the supposed *Pseudomonas* RTF to sensitive strains of *P. aeruginosa*.

Similarly, Lebek (83) showed that an *E. coli* strain could transfer its RTF to drug-sensitive *P. aeruginosa* strains at low frequency.

From these studies, it must be concluded that the transfer of drug resistance between enterobacteria and *P. aeruginosa* may take place in nature. However, it should be pointed out that there is as yet insufficient evidence to prove that RTF is involved in this transfer, although the circumstantial evidence suggests this to be likely.

The only direct physical evidence for extrachromosomal genetic units in *Pseudomonas* comes from the work of Mandel (98), who showed that certain strains of *P. stutzeri* have a satellite band of DNA similar in appearance to the satellite bands of *Proteus*, where a plasmid is known from genetic evidence to be present (43). By growing strains of *P. stutzeri* possessing this satellite band in nutrient broth containing 25 μg of neutral acriflavine per ml, bacterial populations resulted in which the satellite DNA was absent.

Radiation Sensitivity

Pseudomonas is of special interest in radiation biology because it displays an extremely high sensitivity to ionizing radiation. A start has been made on the study of the genetic basis of radiation sensitivity in *P. aeruginosa* (87). The two strains PAO1 and PAT2 were selected for study and were shown to differ in their sensitivity to UV radiation. Crosses between auxotroph derivatives of these strains have shown that the genetic control of sensitivity to UV radiation in these strains is multigenic. Mutants with altered radiation response were isolated after mutagen treatment (87). In addition, mutants lacking host cell reactivation (HCR) were isolated and showed properties similar to such mutants of other organisms (61).

One area of particular interest is the relationship between genes which affect sensitivity to radiation and those which affect recombination. It was shown that UV irradiation of transducing phage affects the degree of linkage when such irradiated phage is used for cotransduction studies. Whereas UV acts to reduce cotransduction frequency, ionizing radiation has no effect on the degree of linkage (72). These effects of UV are more marked when an *hcr* mutant is used as the recipient in transduction (Holloway, *unpublished data*).

The Les$^-$ mutants of *P. aeruginosa* described earlier are unusual when compared with the *E. coli rec*$^-$ mutants, in that they are only slightly

more sensitive to UV irradiation than the wild type in terms of their survival curves. In contrast, the *E. coli rec*$^-$ mutants are highly radiation sensitive (25). However, it is known that when *P. aeruginosa* Les$^-$ strains are made lysogenic for an inducible temperate phage, they do not show induction when irradiated with UV (136). Strain PAO1 (from which the Les$^-$ mutants were made) is aeruginocinogenic, and this aeruginocin is UV inducible. Thus, it is likely that the normal radiation sensitivity of this strain is due to two properties—a sensitivity caused by the effect of UV on DNA, and an effect caused by the induction of the aeruginocin. In the Les$^-$ strain, this latter mechanism may not operate, hence the apparent radiation resistance of these Les$^-$ strains when compared to such mutants in *E. coli*.

Genetics of Psychrophily

P. aeruginosa is a mesophilic bacterium. Olsen and Metcalf (110) showed that mutants of *P. aeruginosa* can be obtained which are psychrophilic and have acquired the ability to grow at temperatures near 0 C. The doubling time at 3.5 C was 4 to 6 hr, which is comparable to that of wild-type psychrophilic strains of other *Pseudomonas* species. A transduction analysis of the genetic basis of these mutants suggested that a limited number of genetic loci was involved, and cotransduction with tryptophan independence was demonstrated for some. It can be concluded that the relation of the cell division rate to temperature is not dependent upon a large range of temperature-sensitive enzymes but rather on the products of only a few genes. A knowledge of the genetic basis of temperature dependence of growth is necessary for *Pseudomonas*, in view of the industrial significance that this organism has for the decomposition of cold-stored foodstuffs and products of medical importance, such as whole blood.

SUMMARY

For many years, ever since the investigations of Gessard on the blue pus organism in 1882 and those of den Dooren de Jong on the saprophytic pseudomonads, the genus *Pseudomonas* has provided a fruitful and interesting subject for biological research. At the present time, not only is it important academically because of its biochemical and metabolic abilities, but also—through its innate resistance to antibiotics—*P. aeruginosa* is achieving a notorious position in medical microbiology. At the industrial level, pseudomonads are increasing in importance as an agent of spoilage.

Our genetic knowledge of this organism is providing new tools for the solution of these problems. Bacteriophage studies have contributed extensively to these solutions, particularly with the transducing phages now available. Much remains to be done, however, before the mechanism of conjugation is sufficiently well understood to permit its use in extensive mapping.

The transduction analysis of linkage has revealed an important difference in gene distribution compared with what is known of other bacteria, and the current study of control mechanisms in *Pseudomonas* suggests a relationship between the distribution of genes and the way in which they are controlled. The further elaboration of recombination techniques for *P. putida* will enable a much better understanding of the mechanisms by which this and other species can catabolize such a wide range of substrates. There is no denying the tremendous intellectual stimulus provided to biological research by the operon model; nevertheless, as pointed out by Clarke and Lilly (27), perhaps it has tended to stifle originality in discovering other mechanisms of regulation. It is possible that the *Pseudomonas* pattern of genetic arrangement and control may represent a picture closer to that occurring in higher organisms, including man.

Obviously, much remains to be done. New tools are needed, such as the development of a partial diploid structure, analogous to an *E. coli* F′. Other sex factors must be investigated with a view to a further understanding of the mechanism of conjugation, and this information must in turn be used to assess the number, size, and structure of the genetic units comprising the *Pseudomonas* genome. The role of bacteriophage genome in the overall genetic structure of *Pseudomonas* needs to be investigated. At the practical level, the nature of the resistance of this organism to so many deleterious agents remains to be fully understood. The solutions to these and other problems are likely to be of wide genetic significance.

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