A MAP ORDER FOR *HIS I*, ONE OF THE GENETIC REGIONS CONTROLLING HISTIDINE BIOSYNTHESIS IN *PSEUDOMONAS AERUGINOSA*, USING THE TRANSDUCING PHAGE F116

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A previous communication (MEE and LEE 1967) described the use of two-point crosses in a transduction analysis of histidine requiring mutants of *Pseudomonas aeruginosa*. It was shown that histidine biosynthesis is controlled by at least five genes or groups of genes which have been designated *his* I, *his* II, *his* III, *his* IV and *his* V. However, no fine structure mapping has been carried out in *Pseudomonas aeruginosa*. This paper presents a map order for the *his* I region based on a transduction analysis using three-point crosses.

MATERIALS AND METHODS

Media and general cultural procedures: The media and general cultural procedures are those of MEE and LEE (1967).

Bacterial strains: In our previous paper we allotted numbers to the histidine loci which did not completely conform to the proposals outlined by DEMEREC *et al.* 1966. These anomalies have been corrected and now conform with the proposed locus nomenclature of DEMEREC *et al.* The strains which are derivatives of *Pseudomonas aeruginosa* 1C (HOLLOWAY 1955), are listed in Table 1.

Bacteriophage strain: F116 (HOLLOWAY, MONK, HODGINS, and FARGIE 1962).

Transduction experiments: The method previously described (MEE and LEE 1967) has been modified slightly. Prior to use, phage preparations were irradiated with 600 ergs/mm^2 of ultraviolet light (UV). Previous experiments (HOLLOWAY *et al.* 1962; LEE, unpublished) have shown that irradiation of the phage with low doses of UV increases the frequency of F116-mediated transduction. Phage and bacteria were mixed, incubated for 20 min to allow adsorption and then the bacteria were sedimented by centrifugation, resuspended and aliquots were added to MM agar layers and poured over met MM agar plates. The recombinants which arose after 48 hrs incubation were streaked on met MM and then were spot tested onto MM agar.

RESULTS AND DISCUSSION

In *Pseudomonas aeruginosa*, the maximum transduction frequency using the bacteriophage F116, for most of the *His*$^-$ loci examined, is of the order 1 per 10^6 recipient cells (MEE and LEE 1967). This holds if the donor phage is prepared on the wild-type strain or on a strain with an unlinked auxotrophic marker. If, however, the donor is prepared on a *his* I mutant and transduced into another *his* I recipient, the frequency of transduction is reduced more than ten fold to 1 per 10^7 or 1 per 10^8 recipient cells. It was, therefore, impossible to determine the exact order of the *his* I alleles from two-point intragenic crosses (prototroph

TABLE 1

Bacterial strains used in this study

Mutant No.	Histidine locus No.	Description
PAO 7	*his-1*	methionine histidine requiring double mutant obtained from B. W. HOLLOWAY
GMA 137	*his-1*	histidine requiring recombinant derived from PAO 7
GMA 159	*his-9*	cysteine histidine requiring double mutant
GMA 140	*his-9*	histidine requiring revertant of MH 9
GMA 152	*his-32*	adenine histidine requiring double mutant
GMA 156	*his-49*	adenine histidine requiring double mutant
GMA 142	*his-52*	histidine requiring recombinant derived from PAO 300
PAO 300		methionine, isoleucine and valine, arginine, histidine requiring multiple mutant obtained from B. W. HOLLOWAY
GMA 143	*his-56*	histidine requiring recombinant derived from PAO 301
PAO 301		methionine, tryptophan, histidine requiring multiple mutant obtained from B. W. HOLLOWAY
GMA 026	*his-5026*	histidine requiring mutant
GMA 027	*his-5027*	histidine requiring mutant
GMA 033	*his-5033*	histidine requiring mutant
GMA 034	*his-5034*	histidine or adenine requiring mutant
GMA 048	*his-5048*	histidine requiring mutant
GMA 050	*his-5050*	histidine requiring mutant
GMA 053	*his-5053*	histidine requiring mutant
GMA 067	*his-5067*	histidine requiring mutant
GMA 255	*his-5067*	cysteine histidine requiring double mutant derived from *his-5067*
GMA 070	*his-5070*	histidine requiring mutant
GMA 072	*his-5072*	histidine requiring mutant
GMA 074	*his-5074*	histidine requiring mutant
GMA 075	*his-5075*	histidine requiring mutant
GMA 253	*his-5075*	cysteine histidine requiring double mutant derived from *his-5075*
GMA 083	*his-5084*	histidine requiring mutant
GMA 087	*his-5087*	histidine requiring mutant
GMA 090	*his-5090*	histidine requiring mutant
GMA 703	*his-4982*	cysteine histidine requiring double mutant
GMA 710	*his-4982*	histidine requiring revertant from GMA 703
GMA 707	*his-4978*	histidine requiring mutant
GMA 709	*his-4977*	histidine requiring mutant
GMA 252	*his-4977*	cysteine histidine requiring double mutant derived from *his-4977*
GMA 727	*his-4968*	cysteine histidine requiring double mutant
GMA 744	*his-4968*	histidine requiring revertant from GMA 727
GMA 732	*his-4963*	cysteine histidine requiring double mutant
GMA 741	*his-4963*	"leaky" histidine requiring revertant from GMA 732
GMA 735	*his-4960*	cysteine histidine requiring double mutant
GMA 742	*his-4960*	histidine requiring revertant from GMA 735

* This nomenclature follows Demerec et al., 1966.

formation), even when the variables affecting the transduction process were adjusted by using an internal "standard." However, the mutant loci were able to be ordered by a method based on the three point cross using the locus *cys-5242*.

cys-5242, (previously *met-5242*) has been shown to be cotransducible at a frequency of approximately 75% with the *his* I region of the Pseudomonas genome (MEE and LEE 1967 and Table 2). A transduction analysis of cysteine and methionine requiring mutants of *P. aeruginosa* strain 1C has shown that seven groups of mutants can be defined (LEE *et al.*, in preparation). These groups have been called *met* I, *met* II, *met* III and *cys* I, *cys* II, *cys* III, *cys* IV. Although these designations have no real significance outside the laboratory, they are convenient and will be used until *in vitro* assays determine the enzymatic basis of each transduction group. *cys-5242* has been allotted to the group *cys* II. Such cysteine requiring strains were mutated by treatment with N-methyl-N′-nitro-N-nitrosoguanidine and Cys$^-$ His$^-$ double mutants obtained. The histidine loci of these double mutants were examined by transduction and those which were *cys* II *his* I double mutants (GMA 159, GMA703, GMA727, GMA732, GMA735) were used in the three-point crosses. Similarly, four *his* I strains, namely *his-4977*, *his-5050* *his-5067* and *his-5075* were treated with nitrosoguanidine and *his* I *cys* II double mutants obtained.

All the double mutants so derived were checked for cotransduction of the two loci on MM agar and met MM agar using as donor, phage prepared on wild type. The recombinants from methionine supplemented plates were scored for the unselected *cys*$^-$ allele. The results are presented in Table 2. The strains were analysed at different times and the variation in prototroph production was the result of variations in the transducing efficiency of the different phage preparations.

The general method used to order the *his* I mutants was based on the three-point reciprocal cross (DEMEREC and HARTMAN 1956; CLOWES 1958; GROSS and ENGLESBERG 1959). Twenty six independently isolated *his* I mutants were used as recipients and nine *his* I *cys* II double mutants were used as donors in transduction tests. The crosses were performed on met MM agar and His$^+$ recombinants were scored by replica plating onto MM agar for the unselected *cys*$^-$ marker.

The results of the crosses are given in Table 3 as the fraction Cys$^-$/Cys$^-$ + Cys$^+$ for each cross. If little or no integration of the *cys*$^-$ allele was observed in the recombinants, the *his*$^-$ allele of the recipient was assumed to be located distal to the *his*$^-$ allele of the donor as outlined diagrammatically

donor	*cys*$^-$	*his*$^-$	+
recipient	+	+	*his*$^-$

If on the other hand, integration of the *cys*$^-$ allele was observed as the preponderant class of recombinants, the *his*$^-$ allele of the recipient was assumed to be located between the two donor alleles as outlined diagrammatically

donor	*cys*$^-$	+	*his*$^-$
recipient	+	*his*$^-$	+

TABLE 2

Transduction of the doubly marked strains with transducing phage prepared on the wild-type strain

	Number of transductants on MM agar	Ratio $\frac{Cys^+}{Cys^+ + Cys^-}$ on met MM agar	Percent cotransduction
GMA 159 *his-9* *cys-5242*	53	$\frac{41}{54}$	76
GMA 735 *his-4960* *cys-5245*	170	$\frac{171}{193}$	88
GMA 703 *his-4982* *cys-5245*	163	$\frac{149}{167}$	89
GMA 252 *his-4977* *cys-5604*	6	$\frac{21}{29}$	72
GMA 727 *his-4968* *cys-5245*	89	$\frac{99}{106}$	85
GMA 255 *his-5067* *cys-5607*	40	$\frac{42}{49}$	86
GMA 732 *his-4963* *cys-5245*	216	$\frac{218}{250}$	87
GMA 253 *his-5075* *cys-5605*	178	$\frac{210}{231}$	91
GMA 745 *his-5050* *cys-5651*	165	$\frac{95}{120}$	79

Hence, the order of the *his* I alleles was based on a significant incorporation of the *cys*$^-$ allele and not the frequency of prototroph formation. The proposed order of the *his* I mutant sites is shown in Figure 1.

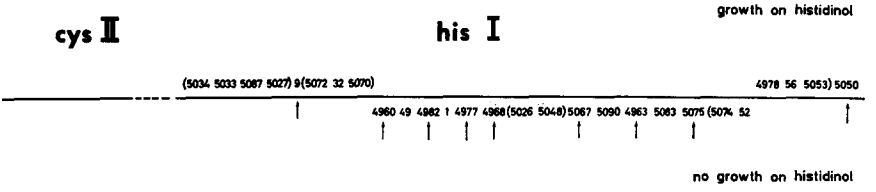

FIGURE 1.—A proposed map order for the *his I* region. The histidine alleles are indicated by their locus number. The map has not been drawn to scale. The arrows indicate those alleles which were used as recipients as well as donors. When acting as donors, strains carrying these alleles also contained a closely linked allele for cysteine biosynthesis. The results of growth tests on histidinol are also presented. Strains shown above the line grow on histidinol and have varying histidinol dehydrogenase activities. Strains shown below the line do not grow on histidinol and show no activity for the enzyme histidinol dehydrogenase.

TABLE 3

The value of the ratio Cys⁻/Cys⁻+Cys+ among transductants arising from crosses between different his *I mutants using a* cys *II allele as the unselected marker*

Recipients	Reversion frequency	Donors *his-9 cys-5242*	*his-4960 cys-5245*	*his-4982 cys-5245*	*his-4977 cys-5604*	*his-4968 cys-5245*	*his-5067 cys-5607*	*his-4963 cys-5245*	*his-5075 cys-5605*	*his-5050 cys-5651*
his-5034	10	5/29	32/56	39/62	17/26	12/39	11/32	34/49	19/45	68**/168
his-5033	15	15**/57	26/56	22/67	63**/131	6/32	35**/85	22/38	97/125	27/46
his-5087	34	6**/71	4/21	10/35	4/13	10/83	13/18	10/29	13/20	7**/99
his-5027	1	16**/19	9/16	11/22	7/12	3/5	21/25	13/20	16/21	26/31
his-9	3	0***/9	12/24	28**/47	15**/41	7/17	3/8	25/42	20/22	33/53
his-5072	6	0**/19	6/24	19/41	29***/73	11/27	21**/48	22/44	55/80	56/84
his-32	8	0/10	10/25	29/45	5/17	7/23	11/23	27/42	44/52	16/38
his-5070	13	0**/60	6/10	18/31	25**/69	15/30	20/26	28/40	27/38	25/39
his-4960	9	2/23	0	11/14	10/22	6/10	7/10	13/17	40/64	26/37
his-49	5	0/10	0**/10	5/7	10**/25	0/3	9/13	22/30	6/15	12/26
his-4982	8	1/14	0/5	1**/39	7***/19	7/13	16/20	23/33	8/17	12/30
his-1	5	0/18	0/8	0/2	4/9	15/19	14/21	11/18	10/21	18/29
his-4977	19	0/29	0/55	0/25	0/14	4/95	4/84	3/63	13/133	17/37
his-4968	15	4/60	3/26	1/20	1/19	0/18	7/10	14/28	32/47	7/28
his-5026	2	0/22	2/18	0/4	0**/2	4**/19	13**/22	14/27	12/28	16/28
his-5048	3	0/16	2/16	2/7	0/11	2**/5	7**/7	15/24	14**/18	25/48

TABLE 8—Continued

Recipients	Reversion frequency	Donors *his-9 cys-5242*	*his-4960 cys-5245*	*his-4982 cys-5245*	*his-4977 cys-5604*	*his-4968 cys-5245*	*his-5067 cys-5607*	*his-4963 cys-5245*	*his-5075 cys-5605*	*his-5050 cys-5651*
his-5067	4	1/32	0/23	2/23	1/28	0/9	0	8/13	16/18	26/31
his-5090	15	0/37	5/45	1/34	0/23	0/1	1/8	15/32	7/18	38/70
his-5083	1	0/10	2/39	0**/43	0/4	0/6	0**/15	0**/3	11/30	11/15
his-5075	5	1/57	3/20	0/37	0/60	0/11	0/41	0/6	0/6	6**/18
his-5074	10	0/87	0/22	1/42	2/93	0/16	0/23	0/10	0***/28	13/18
his-52	24	2/36	0/26	0/29	1/28	0/48	0/16	0/21	0/10	9/20
his-4978	2	0/18	0/23	0/8	0/13	0/6	0/19	2/29	0/18	9/12
his-56	0	0/34	1/50	0/75	1/20	0/34	0/10	0/34	0/5	7/8
his-5053	1	0/34	2/66	1/66	1/45	0/18	1/26	1/44	0/20	8/12
his-5050	0	1/34	2/54	1/34	0/29	0/4	0/17	0/11	0***/20	0

The mutants were ordered by crosses of the type:

donor *cys* II⁻ *his* I⁻ × recipient *his* I⁻

If the order of the alleles was

donor	*cys*⁻	+	*his*⁻
recipient	+	his⁻	+

integration of the *cys*⁻ allele was observed at a measurable frequency.

If the order of the alleles was

donor	*cys*⁻	*his*⁻	+
recipient	+	+	*his*⁻

integration of the *cys*⁻ allele would require a quadruple crossover and the *cys*⁻ allele was rarely detected.

The mutants have been arranged in the proposed order and the reversion frequency of each recipient (per 10^9 cells) is listed. The reversion values for each recipient have not been subtracted from the results. ** (***) indicates the cumulative total from two (three) similar crosses.

Therefore, the order of the *his* I alleles, which was derived from the results in Table 3, was unaffected by the frequency of prototroph formation and, in most cases, by the frequency of reversion of the recipient. For example, in the first case, a high frequency of reversion of the recipient would increase the denominator value of the fraction (Cys^-/Cys^-+Cys^+). Since this fraction was already

small, any increase in the denominator would have little effect on its value and consequently no effect on the ordering of the alleles. In the second case, any increase in the denominator value due to reversion of the recipient would also tend to reduce the value of the fraction, and could have some effect on the ordering of the alleles.

Six recipients (*his-5033*, *his-5087*, *his-4977*, *his-4968*, *his-52* and *his-5090*) were found to have fairly high reversion values. However, in only two of these (*his-5087* and *his-4977*) was it found that this reversion significantly decreased the fraction $Cys^-/Cys^- + Cys^+$. It was still possible however, to order these two mutants because both had significant incorporation of the *cys*$^-$ allele in crosses with the marker histidine alleles. The map derived from the results of these crosses was consistent and linear. Therefore, although the reversion frequency for each recipient has been included in Table 3, the data have not been corrected for reversion.

The nine *his* I alleles for which *his* I$^-$ *cys* II$^-$ double mutants exist, were able to be ordered unequivocally by reciprocal three-point crosses. However, Table 3 contains only those results obtained as already described. The reciprocal crosses, in fact, do not provide any more information than that which can be obtained by the techniques just outlined. Regarding these strains as marker alleles, the remaining *his* I alleles can be ordered either to the left or to the right of these markers. Thus a total of about eighteen strains can be reliably ordered. In those cases in which there are several *his* I alleles between the marker alleles, the map order can be deduced from the relative frequency of prototroph formation (denominator value of Table 3). Consequently, four sections of the map order indicated by the brackets are still tentative.

The data from crosses involving homologous phage, the "selfed" cross (DEMEREC 1962) were performed as an internal control and were not used in the derivation of the map order. The crosses were performed with the histidine marker alleles only and the recombinants were checked for the Cys$^-$ phenotype. Very few of the recombinants from the "selfed" crosses (1/86) incorporated the *cys*$^-$ allele and therefore could not be distinguished from revertants of the recipient histidine allele.

All the strains presented in Table 1 were effective recipients or donors except *his-4963*. This histidine allele is very leaky and at 20°C shows almost no requirement for histidine. At 37°C, the histidine requirement can be seen in overnight incubation. However, 48 hrs incubation was necessary for the transductants to form colonies and during this time any colonies formed were indistinguishable in the "leaky" background. Acting as a donor, GMA732 (*his-4963 cys* II) produced two types of colonies which were readily distinguishable in all the recipients used. One type was the normal transductant which was either Cys$^+$ or Cys$^-$ and the other type was a small colony which grew poorly during overnight incubation in streaks as outlined in MATERIALS AND METHODS. These colonies, always much more numerous than the former type on the transduction plates, had incorporated the "leaky" *his-4963* allele and the results from these colonies have not been included in Table 3.

TABLE 4

The cotransduction index for each donor his *allele*

Cotransduction indices (Σ Cys$^-$/Cys$^-$+Cys$^+$) have been calculated for each donor. In the case where reversion of the recipient was large enough to decrease the average value of the ratios in Table 3 (*his-5087* and *his-4977*) the results were not included in the cotransduction indices presented.

Donor	$\Sigma \frac{Cys^-}{Cys^- + Cys^+}$	Cotransduction Index (percent)
GMA 159	$\frac{36}{105}$	34.3
GMA 735	$\frac{101}{211}$	47.6
GMA 703	$\frac{182}{336}$	54.2
GMA 252	$\frac{192}{454}$	42.4
GMA 727	$\frac{89}{218}$	40.8
GMA 255	$\frac{195}{350}$	55.7
GMA 732	$\frac{306}{497}$	61.6
GMA 253	$\frac{434}{659}$	67.5
GMA 745	$\frac{410}{707}$	58.0

A value (Σ Cys$^-$/Cys$^-$+Cys$^+$ $\times$ 100) has been calculated for each donor against every recipient and for each recipient against every donor. We have called this value the *cotransduction index*. In Table 4, the cotransduction index is represented for each of the donor strains. The values of the cotransduction index for each recipient did not differ significantly from each other when reversion of the recipient was taken into account. However, the cotransduction index for each donor was characteristic and seems to be related to the position of the donor alleles on the proposed map. Lower values than would be expected were found for the cotransduction index in those crosses involving the proximal histidine alleles. This may be explained by assuming that because of negative interference, the *cys* II allele was less frequently incorporated in crosses involving the proximal *his* I alleles.

All members of the *his* I group have been tested for their growth response to histidinol. The results together with the proposed map order are presented in

Figure 1. It can be seen that mutants with similar nutritional characteristics tend to group in specific regions of the genetic map. It appears that the mutants can be ordered into three groups: a central group of mutants which do not grow on histidinol; a proximal group and a distal group, both of which grow on histidinol. Cell-free extracts of some of the strains have been assayed for histidinol dehydrogenase by the method of AMES, HARTMAN and JACOB (1963). The Pseudomonas enzyme is very unstable and only qualitative data are available. Mutants *his-49*, *his-1*, *his-5067* and *his-5075* which do not grow on histidinol showed no histidinol dehydrogenase activity. Of the strains which grow on histidinol, a range of activity was found. It seems that strains *his-5033*, *his-5087*, *his-5027*, *his-5070* and *his-56* had normal wild-type levels of activity for histidinol dehydrogenase. The strains *his-5034*, *his-9*, *his-4978*, *his-5053* and *his-5050* had varying degrees of reduced activity; the strains *his-5072* and *his-32* had no *in vitro* activity for histidinol dehydrogenase even though these cells were capable of normal growth on histidinol. Using chromatographic methods outlined by KLOOS and PATTEE (1965), two of the strains which do not grow on histidinol (*his-49* and *his-5026*) were found to accumulate histidinol.

Thus it is possible that a situation similar to that of the *hist-3* region in Neurospora exists in Pseudomonas, that is, a region of the genome codes for a multifunctional enzyme (CREASER, BENNETT and DRYSDALE 1965; CATCHESIDE 1965). In Neurospora, the *hist-3* region codes for three enzymes; those for the second, third and final steps of histidine biosynthesis. The enzymatic functions associated with the two sections of the *his*-I region of Pseudomonas still remain to be determined but it seems they are concerned with step(s) in the first half of the histidine biosynthetic pathway. Thus there appears to be some similarity of the *his* I region of Pseudomonas with the *hist-3* region of Neurospora. This could be confirmed by *in vitro* complementation and enzyme assays, which are being attempted.

CRAWFORD and GUNSALUS (1966) have shown that the regulation of tryptophan biosynthesis in *Pseudomonas putida* is different from that found in other bacteria and ISAAC and HOLLOWAY (1968) have also shown an absense of normal regulatory mechanisms controlling uracil biosynthesis in *Pseudomonas aeruginosa*. These genetic, and regulatory differences between Pseudomonas and other bacteria may be important in considering the place of Pseudomonas in the evolution of microorganisms.

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SUMMARY

Five unlinked groups of genes appear to control histidine biosynthesis in *Pseudomonas aeruginosa*. One of these groups of genes, Group I is cotransducible with a gene for cysteine biosynthesis. Double mutants were constructed which contained a Group I histidine mutation and the cysteine mutation. These were

used in three-point crosses to order twenty-seven *his* I mutants. The crosses were made using the transducing phage F116 and the results indicate that negative interference may be involved in the recombination mechanism. A map order for these *his* I mutants is proposed and the phenotypes of the alleles are examined in relation to the map order.

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