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Gene Order and Co-Transduction in the *leu-ara-fol-pyrA* Region of the *Salmonella typhimurium* Linkage Map

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The gene order and orientation in the *leu-pyrA* region of the *Salmonella typhimurium* linkage map was established by phage P22-mediated transductions. The gene order, in counterclockwise orientation, is *leuO-leuA-leuB-leuC-leuD-ara-fol-pyrA*. The *fol* locus is co-transducible with either the *ara* and *leu* loci or the *pyrA* locus, whereas no co-transduction for the *ara* and *pyrA* loci can be found.

The chromosomes of *Escherichia coli* and *Salmonella typhimurium* display a bipolarity such that some gene clusters (operons) are transcribed in one direction and others in the opposite direction (12, 14, 15). The order of the genes on the linkage maps of *E. coli* and *S. typhimurium* is almost identical (23), with only a few exceptions (3, 16, 25). The genes involved in leucine biosynthesis and metabolism of L-arabinose are closely linked (10, 13, 27). In *E. coli* the gene order *leu-ara-thr* (counterclockwise as the chromosome is usually represented) has been established by co-transduction studies with Plbt phage (8). In *Salmonella typhimurium* the gene order *leu-ara-thr* (counterclockwise) was established by interrupted mating experiments (22). However, the assignment of the relative orientation of the leucine operon and the arabinose system was based on very small differences in the number of Ara$^+$ and Leu$^+$ recombinants. This investigation confirms the above conclusion and establishes the gene order *leu-ara-fol-pyrA* (counterclockwise) through transductional analysis using mutations at the *fol* locus (see Materials and Methods) which can be co-transduced with *leu* as well as with *pyrA*. In addition, evidence is presented that transducing particles that carry the *fol* locus are heterogeneous in their genetic composition.

MATERIALS AND METHODS

The gene symbols used throughout this investigation are those of the 4th edition of the Salmonella linkage map (24). The media and procedures for phage P22-mediated transductions used in this work have been described previously (13). A multiplicity of about 15 plaque-forming units per bacterium was used for all transductions. As controls, uninfected recipient bacteria were spread on plates containing the same selective medium that was used in the transductions. The transduction data given in Fig. 1 are corrected for spontaneous revertants found in the corresponding controls. Replica plating onto minimal agar medium containing the appropriate supplements was used to determine the unselected phenotypes of transductant colonies. All mutant strains used are derivatives of *Salmonella typhimurium* strain LT2; most of the leucine auxotrophic mutant strains had been isolated by P. Margolin (13; Margolin, unpublished data). The mutant strain *leuOABCD5111ara* was obtained from J. Calvo (4). Mutations at the *fol* locus confer resistance to trimethoprim, probably owing to an increase in the level of dihydrofolate reductase (R. Berberich and M. Levinthal, Bacteriol. Proc. GP71, 1969). Strains carrying mutations at the *fol* locus were isolated by selecting for spontaneous mutations to trimethoprim resistance (10 μg/ml) in the corresponding parental strains. Bacteria were plated onto minimal agar media containing 10μg of trimethoprim per ml. Colonies that appeared after 24 to 48 h of incubation at 37 C were cloned at least twice by single colony isolation. It is not known whether the mutations have occurred in one or in several genes. Therefore, the symbol *fol-*, followed by the isolation number, is used to designate the different mutations. The strain *ars-1* was kindly supplied by Jun-ichi Ishidsu. The *ars-1* mutation confers arginine sensitivity (6; Ishidsu, J. Annual Report 14, National Institute of Genetics, Japan 1963), and is considered likely to be a mutation in the gene (*pyrA*) for carbamoyl phosphate synthetase (1).

RESULTS

Berberich et al. (R. Berberich and M. Levinthal, Bacteriol. Proc. GP71, 1969) had reported that mutations in at least two unlinked loci can confer resistance to trimethoprim. All *fol-* mutations used in this investigation were found to occur in the same region near the *ara* loci.

The mapping data are based on P22-mediated transductions. Figure 1 outlines the various crosses and shows the co-transduction frequen-

Cross #	Transductions: Recipient	Donor	Selected marker	Number of colonies tested	Percent cotransduction for the region indicated
1	pyrA401 fol-105	x ars-1	pyrA+	258	77.9; 10.5
2	pyrA401 leuA604	x ara9 fol-104	pyrA+	3,459	6.8; 0
3	pyrA401	x leuD798ara fol-101	pyrA+	9,581	6.2; 0.16
	pooling crosses 1 through 3			13,298	6.4
4	pyrA401	x leu-5111ara	pyrA+	1,042	0.58
5	ars-1	x ara9 fol-104	fol-104	5,635	20.1; 19.1; 0.07
6	leuA124	x ara9 fol-104	fol-104	445	12.2; 2.5
7	pyrA401 leuA604	x ara9 fol-104	fol-104	10,972	19.5; 19.0; 4.9; 0
8	pyrA401	x leuD798ara fol-101	fol-101	5,589	9.2; 58.2; 0.40
9	wildtype	x leu-5111ara fol-107	fol-107	3,942	64.5
10	leuD798ara	x pyrA401 fol-105	fol-105	3,221	15.6; 11.1
11	leu-5111ara	x pyrA401 fol-105	fol-105	569	20.9; 2.11
	pooling crosses 5 through 11			5,635 20,351 17,062	20.1 16.2 18.8
12	leuD798ara fol-101	x wildtype	leu+	2,044	4.3
13	leuD798ara	x pyrA401 fol-105	leu+	3,182	5.4; 0
			ara+	1,042	5.6; 0
14	leuD798ara fol-101	x ars-1	leu+	831	1.2; 0
	pooling crosses 12 through 14			7,099	4.6
15	leu-5111ara	x fol-106	ara+	1,115	0.27
			leu+	3,610	0.78
16	leu-5111ara fol-107	x wildtype	leu+	1,107	1.44
17	leu-5111ara	x pyrA401 fol-105	leu+	2,663	1.05; 0
	pooling crosses 15 through 17			8,495	0.88
18	leuA124	x ara9 fol-104	leu+	2,341	31.4; 0.5
19	pyrA401 leuA604	x ara9 fol-104	leu+	5,110	36.9; 0.7; 0
20	pyrA401	x leuD700ara	pyrA+	109,914	leu700ara; 0.09
21	pyrA401	x leuD700ara supQ1	pyrA+	105,737	0.11
	pooling crosses 20 and 21			215,651	0.10
22	leuD700ara	x pyrA401	leu+	24,945	0
23	ara9	x leuD466	ara+	3,133	65.8
24	ara9	x leuA430	ara+	2,579	40.4

FIG. 1. *Mapping data. The deletion leuOABCD5111ara is abbreviated as leu-5111ara. Details are described in Materials and Methods.*

cies for the different regions. Where appropriate, the data of several crosses were pooled to establish the average co-transduction frequency for specific regions. The mutations *ars-1* and *pyrA401* are co-transducible with 77.9% (Fig. 1, cross 1).

Crosses 1 through 4 and 6 through 11 in Fig. 1 show that mutations at the *fol* and *pyrA* loci are co-transducible at frequencies from 6.4 to 16.2% depending which of the two markers had been selected for. Such differences in co-transduction frequencies for the same pair of markers have been described previously (13, 25). Blatt and Umbarger (2) have mapped the *ilvS* gene between *pyrA* and *thr*, and showed that *ilvS* is co-transducible with *pyrA* but not with *fol*. Their value of 5.4% co-transduction between *pyrA* and *fol*, with *pyrA* as selected marker, compares well with the value of 6.4% in crosses 2 and 3 (Fig. 1). Among 14,431 colonies tested (crosses 2 and 7), no co-transductants for *ara9* and *pyrA401* were found; this is in agreement with previously reported data (9) showing no *ara-pyrA* co-transductants among more than 70,000 transductants for a single marker, indicating that the distance between the sites of the *ara9* and *pyrA401* mutation is too big to be contained on one transducing particle. Seemingly contradictory to this are the results of cross 5, in which four colonies (0.07%) showed co-transduction of the *ara9-fol-ars-1* region. Since these four colonies distinguish themselves in three characteristics from the recipient strain, namely inability to metabolize arabinose, resistance to trimethoprim, and resistance to arginine, it is unlikely that they are due to spontaneous events. It is therefore concluded that they are either contaminants or true co-transductants. The latter possibility would indicate that the *ars-1* mutation is significantly closer than the *pyrA401* mutation to the arabinose operon. This is further supported by the relatively high recombination frequency between *ars-1* and *pyrA401* of 22.1% (Fig. 1, cross 1). Eisenstark (6) has reported even higher recombination frequencies of approximately 50% between *ars-1* and several *pyrA* mutations, which cause an arginine and uracil requirement.

Crosses 5, 6, and 7 as well as 18 and 19 in Fig. 1 establish the co-transduction frequency of the *fol* focus with the arabinose (approximately 18.8%) and leucine loci (less than 1% for *leuA* mutations). The co-transduction frequency of 65.8% for the *leuD-ara* and 40.4% for the *leuA-ara* regions (crosses 23 and 24) are in agreement with previously published data (13). These data establish the gene order *leuOABCD-ara-fol-pyrA*. Additional support is obtained from crosses involving the deletions *leuD700ara* (contains a deletion of part of the *leuD* gene) (9), *leuD798ara* (lacks the entire *leuD* gene) (9), and *leuOABCD5111ara* (lacks the entire leucine operon) (4). All three deletions lack the arabinose operon and extend further towards the *fol* locus, as indicated by the significantly higher co-transduction frequencies when the deletion strains are used as donors than when point mutations are used as donors (compare crosses 8 and 9 with crosses 5, 6, and 7). If the deletion strains, however, are used as recipients in transductions, very much lower co-transduction frequencies are obtained (crosses 10 through 17) suggesting that these deletions are relatively large in relation to the size of the DNA of a transducing particle. The deletions extend far enough toward the *pyrA* locus so that the deletion and the *pyrA* locus can be carried in one transducing particle. As reported previously (9), when such a deletion strain, *leuD700ara*, was used as a donor in transductions co-transduction between the deletion and the *pyrA* locus was demonstrated, although with relatively low frequencies of approximately 0.1% (Fig. 1, crosses 20 and 21), whereas no co-transduction was detectable in a reciprocal cross (Fig. 1, cross 22). The marker *supQ1* in cross 21 is a suppressor, which is specific for *leuD* mutations; *supQ* mutations lie near *proA* (9) and thus do not participate in the integration and recombination events in the *leu-pyrA* region. Similarly, low linkage with *pyrA* was found for deletions *leuD798ara* and *leuOABCD5111ara* as donors (Fig. 1, crosses 3, 4, and 8), whereas no linkage was observed when the deletions were recipients (Fig. 1, crosses 13 and 17).

DISCUSSION

Ozeki (18) presented evidence that P22 transducing particles are not formed randomly from the *S. typhimurium* DNA. Since then some genetic heterogeneity has been reported among P22 transducing particles for specific markers (20, 21, see also discussion in 19) although the size of the transducing DNA fragments appears to be uniform. The data presented here provide evidence that the *pyrA* to *leu* region of the *S. typhimurium* chromosome also gives rise to transducing particles which are not of a uniform genetic composition. Transducing particles which carry the *fol* marker contain either the *pyrA* or the *leu-ara* region but never both. However, we still do not know if these transducing DNA fragments are cut out from the bacte-

rial chromosome completely at random or if there are preferential end points.

Recombination and co-transduction frequencies cannot be used as accurate additive measures for the distance between markers, since recombinational events in small regions are not independent of each other and since multiple events in larger regions are generally not detectable. Furthermore, different co-transduction frequencies for the same region have been found in reciprocal crosses and marker specific effects on recombination have been described in many systems (for example, see 17). Nevertheless, especially when larger distances are involved, it would be useful to obtain some approximation of the physical distance between markers. Such a value could give an estimate of the maximum number of cistrons in the region between certain markers if no other genes in that region are known.

In mapping studies by transduction, additional factors can affect the apparent recombination frequency between two markers A and B. If the selection in the transduction was for integration of marker A into the recipient genome, then the frequency of co-transduction with marker B depends not only on the frequency of recombination between A and B, but also on the frequency of marker A transducing particles which also carry the region of marker B. Since the transducing particles for a particular marker are not genetically homogeneous, with increasing distance between A and B the frequency of transducing particles carrying both A and B decreases, yet only such transducing particles can give rise to co-transductants provided that no (or an even number of) crossing over occurred between the sites of marker A and B. Furthermore, as the distance between marker A and B approaches the size of the transducing DNA, co-transduction is additionally reduced owing to the decreasing size of the end regions of the transducing DNA, in which crossing over must occur in order to yield co-transductants.

Assuming the transducing particles are randomly produced and the transducing DNA is of uniform length, approximately 90 to 100% of the length of a P22 phage genome (5, 26), a simple equation can be derived that correlates co-transduction frequency c with the linear distance t between markers A and B. All distances are measured as fractions of the length of the transducing DNA, and it is assumed that the frequency of crossing over is linearly proportional to the length of the region in which the crossing over can occur. If A is the selected marker in the transduction and s the length of the region to one side of the marker A for any specific transducing DNA, then (I) $c_s = s \times (1 - s - t)/s \times (1 - s)$ expresses the likelihood of co-transduction of marker A and B based on the likelihood of transduction of marker A, for that particular transducing particle. The total co-transduction frequency c is obtained by integrating the value c_s (equation 1) for all values of s from $s = 0$ to $s = 1 - t$. This results in the equation (II) $c = 1 - t + t \cdot \ln t$. Wu (28) has published a similar model for three point analysis of random general transduction. The equation (2) has been used to transform the co-transduction frequencies into linear distances, as drawn in Fig. 2, which shows a reasonable degree of additivity for these measurements. The fact that sometimes rather large differences in co-transduction frequencies can be found in the same cross when the selection is for different markers, can be possibly taken as an indication

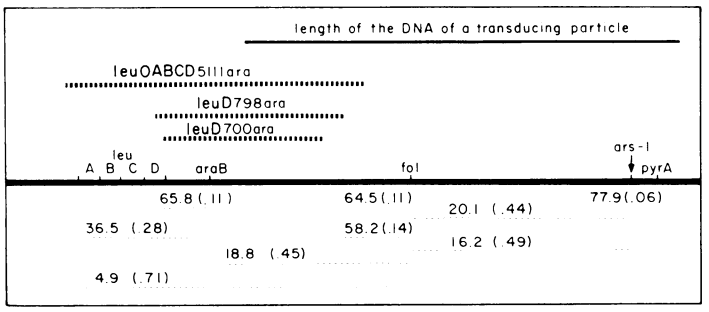

FIG. 2. *Linear distance map of the leu-pyrA region. The boldly dashed lines indicate the extent of three deletions. The values above the lightly dashed lines are the percentages of co-transduction for that region (see Fig. 1); the values in brackets indicate the size of this region, measured as fractions t of the length of the DNA of a transducing particle (see equation 2).*

that the transducing particles are not random and/or that certain classes of transducing particles are more frequent than others, or that crossing-over frequency is not strictly related to distance. For example, see Fig. 1, crosses 2 and 7 show a co-transduction frequency for the region *fol-pyrA* of 6.8% (t = 0.65) when *pyrA* is the selected marker and 19.5% (t = 0.45) when *fol* is the selected marker.

Phage P22 DNA has a molecular weight of 26 million and therefore can accommodate approximately 40 genes (11); the transducing DNA of P22 transducing particles has roughly the same size, so that the size of one average gene is equivalent to a *t* value of 0.025. Using equation (2) and the data of Fig. 1, one can derive an estimate for the distance between the *fol* and *pyrA* loci equivalent to approximately 17 genes. About the same number of genes can be accommodated between the *ara* and the *fol* loci. The deletions *leuD798ara* and *leuOABCD5111ara* extend relatively close to the *fol* locus and delete approximately 12 to 14 genes between the *ara* and the *fol* loci. These deletion strains show only a leucine requirement (and an inability to metabolize arabinose), and therefore the deleted regions must be either silent or contain genes that are not essential under the standard growth conditions.

The data presented unequivocally determine the gene order *leuOABCD-ara-fol-pyrA*. The relative orientation of the genes of the arabinose system, however, is not yet established, since the arabinose operon has not been studied in detail in *Salmonella typhimurium*. Preliminary studies of strains harboring *leuD* deletions that extend towards or into the *ara* region (Kemper, unpublished data) suggest the same gene order as in *Escherichia coli* (6), *leuOABCD-araCOIBAD*.

Mutations at the *fol* locus, conferring resistance to trimethoprim, can be selected easily. They are very useful for strain constructions since the co-transduction with *pyrA* or *ara* and *leu* allows co-integration of known mutations into different strains.

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