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# The pi-Histidine Factor of *Salmonella typhimurium*: a Demonstration that pi-Histidine Factor Integrates into the Chromosome

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The *Salmonella typhimurium* pi-histidine episome was identified by Ames et al. (2) in an unstable partial revertant of a deletion mutation-containing strain, *hisG203*. *HisG203* lacks the histidine operator, promoter, and part of the first structural gene. In this paper, we study some properties of pi factor and demonstrate a low frequency of pi integration into the chromosome at or near the histidine region.

A deletion mutation, *hisG203*, of *Salmonella typhimurium* LT-2 (see Fig. 1) removing the promoter-operator region and extending into the first structural gene of the histidine operon results in the loss of activity of not only the enzyme specified by the damaged *hisG* gene, but also the activity of the enzymes specified by all the other (intact) structural genes of the operon (2). The mutation *hisG203* does not revert to prototrophy either spontaneously or by treatment with mutagenic agents. It does not express the genes *hisD* or *hisC* controlled by the P1 promoter (3). Secondary mutations can be induced enabling *hisG203* to grow on histidinol (HOL; expression of the *hisD* gene).

Ames et al. (2) and St. Pierre (16; M. St. Pierre, Ph.D. thesis, The Johns Hopkins Univ., 1967) described three classes of secondary mutants capable of *hisD* gene expression. Ames et al. (2) studied two of these classes extensively. One class consists of extended deletion mutations removing additional parts of the *hisG* gene on one side and presumably extending the deleted segment of the genome on the other end, connecting it to unknown promoters. The deletion mutations, *his-1302* and *his-1304* (see Fig. 1), are two such examples. A second class appears to be a duplication of part of the undamaged histidine genes of *hisG203*, present in the bacterium as an extrachromosomal piece (pi). Such partial diploids are unstable and segregate HOL nongrowers (Hol$^-$), which are haploid and contain the original *hisG203* lesion. The genes carried by pi are not cotransducible with the histidine region of the chromosome although pi may be transferred from strain to strain by P22-mediated transduction.

A third class of Hol$^+$ revertants, described by St. Pierre (16; M. St. Pierre, Ph.D. thesis, The Johns Hopkins Univ., 1967), are point mutations (represented in Fig. 1 by *hisG1306*) in the undeleted segment of *hisG* which permit the expression of the undamaged genes of *hisG203*. In all three cases, the enzyme activities of these secondary mutants of *hisG203* are no longer under histidine regulation.

The studies reported here provide a genetic map of the ends of the pi piece and demonstrate that pi is an episome capable of integrating within or near the histidine region of the *Salmonella* chromosome.

## MATERIALS AND METHODS

**Media.** E minimal medium of Vogel and Bonner was used (17) with 0.2% (w/v) glucose added after autoclaving. Supplements to E medium were as follows: L-amino acids as required, 20 $\mu$g/ml; L-histidinol, 150 $\mu$g/ml; adenosine, 10 $\mu$g/ml; thiamine, 1 $\mu$g/ml; and streptomycin, 1 mg/ml. Enriched minimal medium (EM) consisted of E medium supplemented with 1.25% (v/v) liquid nutrient broth (Difco). Double enriched minimal medium (2EM)

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contained 2.5% (v/v) liquid nutrient broth (Difco). Whenever solid medium was desired, 1.5% (w/v) of Difco agar was added to the above liquid media. Indicator medium was MacConkey agar (Difco) with 1% (w/v) sugar added.

**Bacterial strains.** All mutant strains were isolated from *S. typhimurium* LT-2. A map of the histidine point and deletion mutations is shown in Fig. 1. The genotypes of strains carrying more than one mutation are given in Table 1. The isolation and characterization of pi revertants of *hisG203* has been described (2; M. St. Pierre, Ph.D. thesis, The Johns Hopkins Univ., 1967). The method of isolating TA526 (*his-2327 hisT1504*) has been published (4).

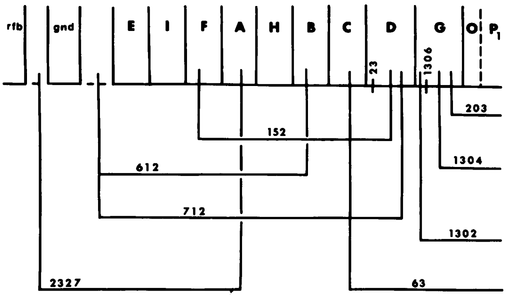

FIG. 1. *A map of the histidine region. Letters G, D, C, B, H, A, F, I, E, correspond to the structural genes of the histidine operon. Letter O denotes the histidine operator and $P_1$ denotes the principle histidine promoter. The gene, gnd, denotes the structural gene for gluconate-6-phosphate dehydrogenase, and the symbol, rfb, denotes the region specifying the O antigen.*

Strains containing pi were constructed from pi revertants of *hisG203* as follows. Bacteriophage grown on *hisG203* carrying pi was used to transduce recipients such as *hisGDC63* to growth on HOL. Transductants, *hisGDC63*[pi], grow on medium containing HOL but not on minimal medium. Transducing particles derived from *his-63*[pi] were used to transfer pi into the various Hfr strains used. Since all the Hfr strains are *hisD*$^-$, the only source of the *hisD*$^+$ allele in a transducing lysate of *his-63*[pi] is the pi factor.

PS108 (*edd-102 gnd-51*) was constructed by the general procedures of Peyru and Fraenkel (10). The deletion mutation, *his-640*, extends beyond the histidine region into *gnd*, the structural gene for gluconate-6-phosphate dehydrogenase. After mutagenesis of *his-640* by *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (Aldrich Chemical Co.) (1), penicillin selection was performed (8) with sodium gluconate as the sole carbon source. The penicillin mixture was plated on MacConkey agar containing sodium gluconate. White gluconate nonfermenting colonies were chosen. One of these, PS95 (*his-640 edd-102*), was deficient in Entner-Douderoff dehydrase. Strains mutant in both the *gnd* gene and the *edd* gene are phenotypically gluconate nonfermenting (Glk$^-$). Phage grown on wild type was used to transduce PS95 to His$^+$ while also donating the *gnd*$^+$ allele. The resultant strain, PS97, is Glk$^+$ and His$^+$ but genetically still *edd-102*. Another cycle of mutagenesis and penicillin selection resulted in PS108 (*edd-102 gnd-51*) which is His$^+$ and Glk$^-$.

PS113 (HfrK9 *thrA8 hisD3940*) was isolated after *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine mutagenesis (1) of SA654. Penicillin selection (8) was carried out in E medium containing threonine at 42 C, and the penicillin mixture was plated on EM containing histidine at 42 C. Mutations in the *hisD* gene (Hol$^-$)

TABLE 1. *Multiply mutant bacterial strains*

| Strain no. | Genotype[a] | Source |
|---|---|---|
| PS39 | HfrB2 *gal-50 hisD23*[pi-41] | See Materials and Methods |
| PS66 | Pi-41 *hisD23 strA101*[b] | See Results |
| PS108 | *edd-102 gnd-51* | See Materials and Methods |
| PS113 | HfrK9 *hisD3940 thrA8* | See Materials and Methods |
| PS130 | *purG909 his-63 strA101* | See Materials and Methods |
| PS157 | HfrB3 *gal-50 hisD23*[pi-2] | See Materials and Methods |
| PS158 | HfrK9 *thrA8 hisD3940*[pi-2] | See Materials and Methods |
| PS159 | *his-644 sup-521* | *lac*$^-$ Segregant of TA221 |
| SA654 | HfrK9 *thrA8* | Kenneth Sanderson |
| SB465 | HfrB2 *gal-50 hisD23*[pi-2] | See Materials and Methods |
| SB485 | *his-63 strA101* | Philip E. Hartman |
| SB488 | HfrA *purD222 gal-50 hisD23*[pi-2] | See Materials and Methods |
| SB533 | *his-63 pro-722 strA101* | Philip E. Hartman |
| SB1130 | *his-57*[pi-2] | Philip E. Hartman |
| SU115 | HfrA *purD222 gal-50 hisD23* | Kenneth Sanderson |
| SU219 | HfrB2 *gal-50 hisD23* | Kenneth Sanderson |
| SU250 | HfrB3 *gal-50 hisD23* | Kenneth Sanderson |
| TA221 | *his-644 sup-521*[F'lacZ82] | Bruce Ames |
| TA526 | *his-2327 hisT1504* | Bruce Ames |

[a] The origins of transfer of the Hfr strains and the genetic symbols used are explained in Sanderson's review paper (11).

[b] PS66 has pi-41 integrated into the chromosome (see text). This is indicated by a loss of parentheses.

were identified after replica plating onto minimal agar, minimal agar containing HOL, and minimal agar containing histidine. Strains carrying *hisD* mutations were tested for growth at 42 and at 30 C. PS113 will grow on minimal medium containing HOL at 30 but not at 42 C.

Strain PS130 (*purG909 his-63 strA101*) was isolated from SB485 after *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine mutagenesis (1) and penicillin selection (8). The penicillin mixture was plated on minimal medium containing adenosine and thiamine. Strains carrying purine mutations were identified after replica plating to minimal medium. The allele, *purG909*, was characterized by growth in medium containing inosine and by co-transduction with *purG94*.

**Bacteriophage.** Two bacteriophage, MG40 (which grows on P22 lysogens) (7) and a nonlysogenizing mutant of P22, L4 (15), were used.

**Genetic tests.** The preparation of transducing lysates of P22-L4 and MG40 and the use of these lysates for transduction have been described (7, 15).

Conjugation experiments were performed by the method of Sanderson (*personal communication*). The donor was prepared by diluting an overnight nutrient broth culture 1:20 with fresh nutrient broth and incubating at 37 C for 3 hr without aeration. One milliliter of donor was added to 2 ml of recipient (stationary phase, overnight nutrient broth-grown culture) plus 2 ml of nutrient broth. The mixture was centrifuged at 4,000 rev/min for 10 min and incubated at 37 C for the times indicated below. Mating was terminated by resuspension of the pellet, appropriate dilution, and agitation with a Vortex mixer (Scientific Products) for 90 sec.

**Acridine curing of pi strains.** The acriflavine curing experiment reported in Fig. 2 was adapted from the experiments of Scaife (12). SB1130 (*his57*[pi-2]) was the pi strain used. This strain will grow on HOL because it contains pi. SB1130 was grown overnight in E medium, diluted 1:$10^6$, and inoculated into 10 ml of nutrient broth, *p*H 7.8, containing acriflavin at the concentrations as indicated in Fig. 2. After growth to saturation, the cultures were appropriately diluted and plated on E medium containing histidine (to monitor the total cell number) and E medium containing HOL (to monitor the number of cells containing pi). The number of colonies appearing on HOL plates divided by the number of colonies appearing on the histidine plates was taken to be the fraction containing pi.

**Enzyme assays.** Bacteria were grown and extracts were prepared as described by Smith and Ames (14). The enzyme, phosphoribosyl transferase (E.C. 4.2.1c), specified by the *hisG* gene, was assayed by the method of Voll et al. (18). The enzyme, cyclase, corresponding to the *hisF* gene, was assayed by the method of Smith and Ames (14). The substrate for the cyclase assay, phosphoribosyl formimino amino imidazole ribonucleotide, was kindly provided by R. F. Goldberger (National Institutes of Health). The activity of gluconate-6-phosphate dehydrogenase was measured by using the procedure of Fraenkel and Horecker (6), as modified by Murray and Klopotowski (9).

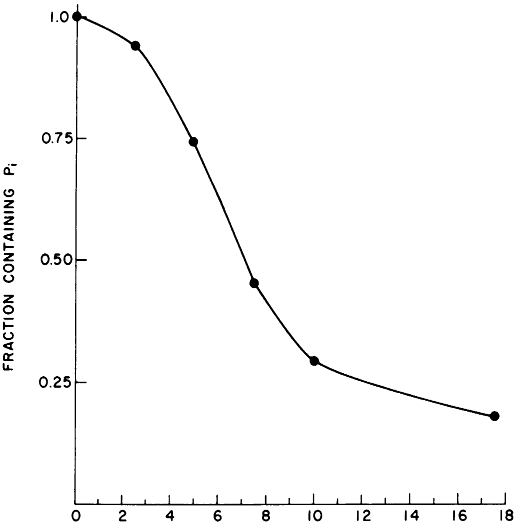

FIG. 2. *The curing of his57[pi2] by acriflavine. See Materials and Methods and Results for experimental technique.*

## RESULTS

**Response for pi factor to acriflavin.** The original strains containing pi were isolated as partial revertants of *hisG203* which expressed *hisD* function. Strains possessing the enzyme histidinol dehydrogenase (specified by the *hisD* gene) can utilize HOL in place of histidine. Bacteriophage P22 grown on strains containing pi could transduce strains carrying *hisD* mutations to growth on HOL. If the transduction recipient was *hisG*$^+$, then the pi transductants were prototrophic, since pi can complement all the histidine genes except *hisG* (2). Both the original strains containing pi and pi strains derived by transduction are unstable, giving rise to large, sectored colonies and small, nonsectored colonies when plated on minimal medium enriched with growth-limiting amounts of histidine (2EM medium) (2). Successive single-colony isolations show that the small nonsectored colonies are stable, haploid, and require histidine, whereas the large, sectored colonies are diploid, contain pi, and give rise once again to large, sectored colonies and small, nonsectored colonies when restreaked on 2EM medium.

Certain extrachromosomal elements can be "cured" by acridine dyes (12). We exposed the pi-containing strain *his-57*[pi-2] to various doses of acriflavine; the dose-response curve is presented in Fig. 2. The loss of pi factor was measured by the loss of ability to grow on

HOL. The results (Fig. 2) show that acriflavine is able to "cure" pi factor. The concentration at which 50% of the cells lose pi, 6.5 $\mu$g/ml, is comparable to that required for 50% curing of the F'*lac*$^+$ episome in *Salmonella* strains (M. Levinthal, *unpublished data*).

**Mapping the ends of pi.** Ames et al. (2) showed that pi factor complemented mutations in all histidine genes except *hisG*. We wanted to see whether (i) pi contains any genetic material corresponding to the *hisG* gene; (ii) pi includes the gene *gnd*, the structural gene for gluconate-6-phosphate dehydrogenase, which maps to the left of histidine (9); and (iii) independently isolated pi factors are genetically homogeneous.

The *hisG* end of pi was mapped by crosses with a series of *hisG* point mutants. Phage were grown on 10 independently isolated pi strains in a *hisG203* genetic background and were used to transduce a *hisGDC63* recipient. Hol$^+$ recombinants were selected. Such recombinants should be diploids containing pi, since *hisG203* cannot transduce the *his-63* deletion to growth on HOL. Diploidy was verified by streaking the Hol$^+$ recombinants onto 2EM plates containing HOL and observing large-sectored colonies and small Hol$^-$ colonies. Phage (either MG40 or L4) were grown on these Hol$^+$ recombinants and used to transduce various recipients containing *hisG* point mutations to prototrophy. Table 2 lists a series of *hisG* point mutants, ordered from top to bottom according to the increased distance of the mutational site from the principal promoter-operator region of the histidine operon. The mutant sites present in *hisG499* and *hisG486* recombined with all the pi elements to produce prototrophic recombinants. The mutant, *hisG1306*, which St. Pierre (16) has shown to be responsible for the third class of Hol$^+$ revertants of the *hisG203* mutation, was the most distal point mutant tested which did not show recombination with the pi factors. The transductants produced by using phages containing pi as a donor appear to be stable recombinants. Twenty prototrophic transductants were purified by single-colony isolation from each successful cross and were streaked onto 2EM medium to test for segregants. No segregants appeared, whereas control strains containing an extrachromosomal pi show many Hol$^-$ segregants and sectored colonies under these plating conditions.

Furthermore, reciprocal transduction, using phage grown on *hisG* point mutants as donors and strains containing pi as recipients and selecting for growth on minimal medium, were unsuccessful, confirming the observation of Ames et al. (2) that it is not possible to construct a prototrophic extrachromosomal pi by transduction.

The other end of one pi factor, pi-2, was shown by Murray and Klopotowski (9) to con-

TABLE 2. *Transductional crosses to determine the hisG end point of pi*

| Recipients | Donors[a] | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | pi-2 | pi-24 | pi-25 | pi-29 | pi-33 | pi-44 | pi-48 | pi-223 | pi-241 | pi-304 |
| *hisG203* | 0[b] | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *hisG119* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *hisG204* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *hisG575* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *hisG618* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *hisG581* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *hisG421* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *hisG460* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *hisG1306*[c] | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *hisG499* | 285 | 112 | 201 | 36 | 119 | 175 | 133 | 98 | 112 | 123 |
| *hisG876* | 184 | 46 | 152 | 12 | 101 | 110 | 52 | 26 | 74 | 68 |
| *his-152* | 970 | 843 | 676 | 233 | 770 | 721 | 679 | 438 | 792 | 811 |

[a] The pi-containing strains are all in a *hisGDC63* genetic background. The pi factors were isolated in *hisG203*-containing strains and the pi elements were transferred by transduction into *hisGDC63*.

[b] The numbers of prototrophic recombinants per transduction plate [selected on plates containing EM agar (E medium supplemented with 1.25%, v/v, Difco liquid nutrient broth)] formed in the cross. The value 0, should be compared to the control value for *his-152* which represents the number of pi factor-containing transducing phage. Thus the 0 value for the cross pi-2 × *hisG203* represents (see the cross pi-2 × *his-152*) $<1$ *his*$^+$ transductant per 970 pi transductants.

[c] *HisG1306* is cold-sensitive. Therefore the crosses involving *hisG1306* as a recipient were performed at 20 C.

tain the *gnd* gene (specifying the enzyme gluconate-6-phosphate dehydrogenase) by the following experiment. The gene, *gnd*, is the nearest known gene distal to the end of the histidine operon and its absence can be determined by assaying the enzyme gluconate-6-phosphate dehydrogenase (6). We repeated the experiment to see if all the pi strains contain the *gnd*$^+$ gene. The relative specific activities (Table 3) of the histidine biosynthetic enzymes and glucose-6-phosphate dehydrogenase of normal repressed wild-type cells are expressed at 1.0. A strain containing pi-41 has a constitutive enzyme level of 1.4-fold that of wild type for all the histidine enzymes whose genes are carried on the pi episome (2). Strains carrying the *hisT1504* mutation are constitutive for the histidine operon and contain enzyme at a level 12 times higher than the repressed wild-type level. We obtained the double mutant strain *hisT1504 hisHAFIE gnd2327* and compared several enzyme activities of haploid *hisT1504 his-2327* with a diploid containing pi-41. Table 3 shows that *gnd*$^+$ is included in the pi-41 factor. Nine other independently isolated pi strains (pi-2, -24, -25, -29, -33, -48, -223, -241, -304) were similarly tested and shown to contain the *gnd*$^+$ gene.

We confirmed the above result by using a different experimental technique. Peyru and Frankel (10) have shown that *Escherichia coli* shows a gluconate nongrowing phenotype (Glk$^-$) when strains are mutant in two unlinked genes, *gnd* and *edd* (the structural gene for Entner-Douderoff dehydrase). Transduction of either mutant allele to wild type produces a Glk$^+$ phenotype. We constructed a *hisD*$^-$ derivative of a *gnd edd* double mutant, as described above, and used it as a recipient in transduction tests with phage grown on pi-containing derivatives of *his-2327*. His$^+$ transductants were selected and tested for the Glk$^+$ phenotype. All His$^+$ transductants were Glk$^+$, and, when His$^-$ segregants were selected, they were all Glk$^-$. Similarly when Glk$^-$ segregants were selected (by streaking on MacConkey agar containing 1% sodium gluconate), all the Glk$^-$ segregants were His$^-$.

TABLE 3. *The assay of a pi-containing strain for gluconate-6-phosphate dehydrogenase (GND)*

| Strain | Relative specific activity | | |
|---|---|---|---|
| | GND | *hisG*[a] | *hisF*[a] |
| Wild type | 1.0 | 1.0 | 1.0 |
| *his-2327 hisT1504* | 0 | 12.8 | 0 |
| *his-2327 hisT1504*/pi-41 | 1.42 | 12.3 | 1.51 |

[a] *HisG* and *hisF* denote the activity of the enzyme specified by these genes. The assays and enzyme preparations are outlined in Materials and Methods.

**Hfr transfer of pi.** Various experiments (2) have shown that a pi element can be transferred to a strain which does not contain pi by transduction with P22 phage grown on a pi-containing strain. We next examined whether pi factor is also transferable by conjugation. For these experiments, we constructed derivatives of two Hfr strains containing pi as described above. HfrA transfers the chromosomal region histidine late, and HfrB2 transfers *his* genes early. Individual selection was made for Pro$^+$ and Hol$^+$. Hol$^+$ (growth on HOL) would indicate transfer of a functional *hisD* gene, i.e., conjugal transfer of pi. The males were eliminated by inclusion of streptomycin in the plating medium. No Hol$^+$ exconjugants were found by using SB488 (HfrA) after 30 min of mating (Table 4), suggesting that pi is not transferred infectiously. The presence of Hol$^+$ exconjugants after 2 hr of mating shows that pi may associate with the donor chromosome during mating and thus be transferred late by *HfrA*. The fact that the Hol$^+$ phenotype was transferred more readily from SB465 (*HfrB2*) than from SB488 (*HfrA*) implies that pi is more closely associated with the histidine region than with the proline region of the donor

TABLE 4. *Linkage of pi genes to the chromosomal histidine region during conjugation*

| Donor | Recipient *hisGDC63, pro-722, strA101* | | | |
|---|---|---|---|---|
| | Selected class[a] | No. of recombinants[b] | | |
| | | *hol*$^+$ | *his*$^+$ | *pro*$^+$ |
| HfrA *hisD23/pi-2* | *pro*$^+$ (30 min)[c] | 0 | 0 | 73 |
| HfrA *hisD23/pi-2* | *pro*$^+$ | 13 | 13 | ~1,000 |
| HfrA *hisD23/pi-2* | *hol*$^+$ | 35 | 35 | 16 |
| HfrB2 *hisD23/pi-2* | *pro*$^+$ | 44 | 44 | 62 |
| HfrB2 *hisD23/pi-2* | *hol*$^+$ | 292 | 292 | 19 |

[a] The *pro*$^+$ recombinants were selected on EM agar [E medium supplemented with 1.25% (v/v) liquid nutrient broth (Difco)] containing 20 μg of histidine per ml. The *hol*$^+$ recombinants were selected on EM agar containing 150 μg of histidinol and 20 μg of proline per ml. The *his*$^+$ recombinants were identified by growth on E agar plates containing 20 μg of proline per ml. All selection plates contained 1 mg of streptomycin per ml.

[b] The number of recombinants per selection plate is given for the selected marker; the number of recombinants also inheriting the unselected genes was determined by transferring the colonies by replica plating to the appropriate medium.

[c] Matings were generally conducted for 2 hr. In this experiment, the mating was interrupted after 30 min by vigorous agitation of the mating mixture by a Vortex mixer.

chromosome. Furthermore, inspection of Table 4 indicates that, irrespective of donor, all recombinants selected for Hol$^+$ (pi transfer) were His$^+$ (pi plus donor chromosomal *hisG*$^+$). The observed linkage requires the cotransfer of the *hisD*$^+$ gene from pi and the chromosomal *hisG*$^+$ gene (Table 5). The linkage between pi and the chromosome was tested in greater detail (Table 5). Further tests using three different Hfr donors containing pi demonstrate the 100% cotransfer of the *hisD*$^+$ gene of pi and the chromosomal *hisG*$^+$ gene (Table 5). This result suggests that during transfer from Hfr strains, pi integrates into the chromosome close to the *hisG* gene.

**Sexual recombinants for Hfr pi-carrying strains.** The tight linkage of pi histidine genes and chromosomal histidine genes during conjugation suggests that the recombinants of conjugation may have pi inserted near the histidine region. To test this possibility, transducing phage were grown on 10 *his*$^+$ exconjugants arising from a cross between a pi-containing male and a recipient carrying *hisGDC63* deletion. The phage lysates were used as donors in a transduction experiment using *his-63* as recipient. The results of one such transduction is shown in Table 6. Phage grown on the conjugational recombinant (PS66) show a 10% cotransduction of the pi *hisD* gene with the chromosomal *hisG* gene (i.e., produce His$^+$ transductants), whereas phage grown on the donor Hfr (hisD23/pi-41) showed no (i.e., less than 0.1%) cotransduction of the pi *hisD* gene with the chromosomal *hisG* gene (*i.e.*, produce Hol$^+$ transductants only). The analysis of the other recombinants showed identical results. These results suggest that integration of pi into the bacterial chromosome is a rare event, that an Hfr cross (demanding pi transfer from donor to recipient) selects for the integrated state of pi, and that when pi does integrate, the site of integration is close to, if not within, the histidine operon.

**PS66, diploid for the hisD gene.** In the previous section, we demonstrated that PS66, a His$^+$ recombinant arising from a cross between a pi-containing Hfr and *his-63*, contained an integrated pi *hisD*$^+$ gene. We now show that PS66 is diploid for *hisD;* it also contains the *hisD23* allele originally present in the Hfr. The allele *hisD23* is an amber mutation (5) and can be suppressed by the amber suppressor contained in TA221. The strain TA221 also contains the deletion *hisGDCHAF644*. Phage grown on PS66 was crossed by transduction into TA221 (*his-644*, *sup-521*) and into *his-644* lacking an amber suppressor. Phage grown on SU219 (HfrB2 *hisD23*) was used as a control. The results in Table 7 show that when *his-644* is the recipient only 10% as many His$^+$ recombinants as Hol$^+$ recombinants are found. However, when TA221 is the recipient, approxi-

TABLE 5. *Cotransfer by conjugation of pi with the histidine region*

| Donor | Recipient *his-63 strA101* | |
|---|---|---|
| | *hol*$^+$ Recombinants[a] | Per cent *his*$^+$ |
| HfrB2 *hisD23/pi-2* | 971 | 100 |
| HfrB3 *hisD23/pi-2* | 113 | 100 |
| HfrA *hisD23/pi-2* | 35 | 100 |

[a] hol$^+$ Recombinants were selected on E agar medium containing 150 μg of histidinol and 1 mg of streptomycin per ml. The Hol$^+$ recombinants were transferred by replica plating to E agar medium to ascertain the presence of the *his*$^+$ genes.

TABLE 6. *The integration of pi near the histidine region*

| Recipient | Selection[a] | Donor phage grown on | |
|---|---|---|---|
| | | Pi recombinant, PS66, from conjugation[b] | Pi recombinant from transduction[c] |
| *hisGDC63* | Hol$^+$ | 460 | 1,069 |
| *hisGDC63* | His$^+$ | 51 | 0 |

[a] Hol$^+$ recombinants were selected on E agar medium containing 150 μg of histidinol per ml. His$^+$ recombinants were selected on E agar medium.

[b] The donor phage was grown on a *his*$^+$ recombinant resulting from a mating between HfrB2 *hisD23/pi-2* and F$^-$ *his-63.*

[c] The donor phage was grown on HfrB2 *hisD23/pi41.* This strain was constructed by transduction using phage grown on *his-712/pi-41* to transduce HfrB2 *hisD23* to a prototrophic diploid.

TABLE 7. *PS66 is diploid for the hisD gene*

| Recipients | Donor phage grown on | | | |
|---|---|---|---|---|
| | *PS66* | | *SU219* | |
| | No. of *his*$^+$ [a] | No. of *hol*$^+$ [a] | No. of *his*$^+$ | No. of *hol*$^+$ |
| *his*-644 | 46 | 513 | 0 | 0 |
| TA221 | 619 | 941 | 312 | 304 |

[a] The selection of His$^+$ and Hol$^+$ recombinants is described in Table 6. The numbers of His$^+$ and Hol$^+$ recombinants were counted on separate plates by using identical samples of transducing phage.

mately twice as many Hol$^+$ recombinants as His$^+$ recombinants occur. When the suppressor strain is used, the His$^+$ recombinants are a mixture of both cotransductants between pi *hisD*$^+$ and *hisG*$^+$, and *hisD23 hisG*$^+$, since the *hisD23* mutation can be suppressed. This hypothesis is confirmed by the results with phage grown on SU219 which gives His$^+$ transductants with the suppressor strain as recipient, but not with *his-644* or recipient. To further substantiate this conclusion, we grew phage on 49 of the His$^+$ transductants of TA221 and 10 of the His$^+$ transductants of *his-644* and retested them on TA221 and *his-644* for the ability to give rise to Hol$^+$ transductants. If the transductant contained pi *hisD*$^+$, it should give Hol$^+$ transductants on TA221 and *his-644*. If the transductant contained only the *hisD23* allele, however, it should give Hol$^+$ transductants only on TA221. All 49 of the phage lysates grown on TA221 transductants yielded Hol$^+$ transductants only with TA221 showing that these contained the *hisD23* allele. The 10 transductants from *his-644* yielded Hol$^+$-transducing particles on both strains, showing that these contained the pi *hisD*$^+$ gene.

**Pi integrated into the chromosome.** In the previous sections, we demonstrated that pi is integrated into the chromosomes or exconjugants when pi is transferred by Hfr strains. If pi integration is a rare event, and pi must be integrated into the donor chromosome for transfer to occur, then the frequency of pi transfer from male to female should be far lower than the frequency of transfer of chromosomal histidine genes. The implication here is that the limiting step in pi transfer is integration into the donor chromosome. In the preceding experiments, we could not score the transfer of chromosomal histidine genes independently of the transfer of pi *hisD* because the His$^+$ phenotype required cotransfer of chromosomal *hisG*$^+$ and pi *hisD*$^+$. To make possible the independent scoring of the transfer of pi and the chromosomal genes, we constructed a pi-containing derivative of PS113 as described above. This Hfr strain has a temperature-sensitive chromosomal *hisD* mutation. In a cross between PS113 (pi-2) and *his-63*, the His$^+$ recombinants that grow at 42 C represent cotransfer of pi *hisD*$^+$ and chromosomal *hisG*$^+$. In contrast, after incubation at 30 C, the His$^+$ recombinants also will include recombinants due to cotransfer of *hisD* temperature-sensitive mutation with *hisG*$^+$. Thus, at 42 C we score only pi transfer, whereas at 30 C we score pi transfer and chromosome transfer. The recipient, PS130, also contains a *purG* marker. This gene is located 14 min more distal to the origin of the Hfr than the histidine region, so the number of *purG*$^+$ recombinants is an independent index of the frequency of chromosomal transfer. The results of an experiment with PS130 as the recipient and PS113 [pi] as the donor (where the His$^+$ recombinants were elected on E agar plates containing 10 $\mu$g of adenine and 1 $\mu$g of thiamine per ml and the Pur$^+$ recombinants were selected on E agar plates containing 20 $\mu$g of histidine per ml) show that at 30 C the number of His$^+$ recombinants was 1,458, the number of His$^+$ recombinants at 42 C was 6, and the number of Pur$^+$ recombinants was 983. By either measure (by the number of Pur$^+$ recombinants or by the number of His$^+$ recombinants at 30 C), pi is transferred less than about once for every 200 chromosomal transfers.

## DISCUSSION

The pi-histidine factor has several properties in common with episomes. Pi can exist in two states, extrachromosomal and integrated into the chromosome. The nonintegrated state is characterized by instability (a segregation frequency of 6%), sensitivity to acriflavine, an absence of cotransduction with the histidine region, and an inability to be transferred by conjugation. Upon integration, pi becomes more stable (a segregation frequency of 0.03%, M. Levinthal, *manuscript in preparation*), insensitive to acriflavine (the 0.03% segregation frequency does not change), cotransduces with the chromosomal histidine genes, and is transferred during conjugation. Pi factor differs from the fertility factors in being noninfectious and non-coimmune. We have transferred the F'*lac*$^+$ episome to strains containing pi (M. Levinthal and J. Yeh, *manuscript in preparation*). We find no difference in acceptance or segregation of either extrachromosomal element. Experiments are in progress to test other extrachromosomal elements for coimmunity to pi. Pi factor does not make its host sensitive to male-specific phage, whereas F'*lac* and F'*lac* plus pi-containing strains are sensitive to these phage.

Both states of the pi-factor are diploid, at least for the histidine genes. The integrated state still segregates *his*$^-$ bacteria. More directly, we have recovered both pi alleles and chromosomal alleles from pi-integrated strains.

Pi factors may have unique ends. The 10 independently isolated pi factors we studied all extended from the operator-distal portion of *hisG* through the *gnd* gene. Of course, our tests are not sensitive enough to detect microheterogeneity, but the degree of homogeneity found is not demanded by our selection procedure. At least two possibilities are suggested: an excision dictated by homology and mediated by the *rec* system or a process of special excision involving enzyme recognition analogous to the *int* excision of bacteriophage lambda (13). Another possibility is that pi represents a minimum length of deoxyribonucleic acid required to express the *hisD* gene and to replicate it autonomously. Experiments are in progress to distinguish between the possibilities.

The integration of pi is a rare event. Our data show that pi is transferred by Hfr strains at a $4 \times 10^{-3}$ lower frequency than the chromosomal genes. We interpret this result as showing pi is integrated into 1 of every 250 male chromosomes. We believe that the structure of recombinants arising from crosses of pi-containing males with *hisGDC63* reflects the structure of pi in the integrated male chromosome. The lack of recombination between pi *hisD* and the chromosomal *hisG* substantiates the above hypothesis. The observed (Table 5) lack of recombination between pi and the histidine region is not due to the use of a deletion mutation (*his-63*) in the recipient. The results of Table 5 can be duplicated by using a strain containing two point mutations (*hisG46 hisD1*). Therefore, the extraordinary linkage observed must be due to the structure of the integrated pi strain, which limits recombination within the pi-factor histidine region.

We have some insight into the structure of the integrated pi. The *hisD* gene of pi and the chromosomal *hisG* are not in their normal position, since phage grown on integrated pi strains show only 10% linkage between *hisD* and *hisG*, whereas phage grown on wild-type bacteria show about 90% linkage between these two genes. The linkage value we observe could be due to the distance between the pi *hisD* gene and the chromosome histidine region or could be due to aberrant pairing between the transducing fragment and the recipient chromosome caused by the duplication of the histidine genes in the donor chromosome. A study of the structure of integrated pi strains, especially when performed with genetically marked pi strains, might settle this question.

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