REARRANGEMENT OF THE BACTERIAL CHROMOSOME USING Tn*10* AS A REGION OF HOMOLOGY

FORREST G. CHUMLEY

AND

JOHN R. ROTH

Department of Biology, University of Utah, Salt Lake City, Utah 84112

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ABSTRACT

The transposable tetracycline resistance element, Tn*10*, can serve as a region of homology to promote *rec*-dependent deletion, duplication and directed transposition of bacterial genes. Tn*10* insertions in regions of the chromosome near the histidine operon (*his*) have been isolated and characterized in *Salmonella typhimurium*. When strains are constructed containing two Tn*10* insertions flanking the *his* operon in the same orientation (Tn*10*-*his*-Tn*10*), recombination can occur between Tn*10* sequences resulting in the deletion of the intervening *his* region. The sites of the Tn*10* insertions determine the endpoints of the deletion. In crosses designed to construct strains carrying Tn*10*-*his*-Tn*10*, another class of unstable recombinants arises in which the *his* region exists in tandem duplication, with a Tn*10* insertion joining the duplicated copies (*his*-Tn*10*-*his*). The sites of the parental Tn*10* insertions mark the endpoints of the duplication. When a strain carrying Tn*10*-*his*-Tn*10* is used as a donor of *his*+ in conjugation or P22-mediated transduction, recombinants can arise in which the *his* region has been transposed to the site of any Tn*10* insertion, far from the normal location of *his* in the recipient chromosome. In this manner, the *his* operon has been moved to the site of a *pyrB*::Tn*10* insertion and has been placed on F′ plasmids. At these new locations, the *his*+ character shows the *rec*-dependent deletion of *his*+ expected for a Tn*10*-*his*-Tn*10* duplication. These methods should be generally useful for the manipulation of bacterial genes.

TRANSPOSABLE genetic elements offer many new methods of analysis to the bacterial geneticist (KLECKNER, ROTH and BOTSTEIN 1977; FAELEN *et al.* 1977; CASADABAN *et al.* 1977; CHUMLEY, MENZEL and ROTH 1979). The transposable drug-resistance elements, such as Tn*10*, have been particularly useful (KLECKNER *et al.* 1975). This paper describes the use of Tn*10* as a region of homology (1) to catalyze the formation of deletions and duplications with predetermined endpoints, and (2) to direct the transposition of genes of interest to sites far from their normal location. These chromosome rearrangements serve as new tools for manipulation for the bacterial chromosome and are also interesting demonstrations of the kinds of genetic events that transposable elements might promote during bacterial evolution.

TABLE 1

Bacterial strains used in this study

Strain	Genotype	Method	Recipient	Donor
TT460	*pyrB692*::Tn*10*	H	LT2	NK337
TT513	*zee-2*::Tn*10*	T	*hisOG203*	Pooled random Tn*10* insertions
TT580	*hisO1242 hisDC2236 zee-1*::Tn*10*	T	*hisO1242 hisDC2236*	NK397
TT627	*strA1 pyrC7*/F'ts114 *lac*+ *zzf-20*::Tn*10*	(CHUMLEY, MENZEL and ROTH 1979)		
TT1704	*his-9533*	(H. M. JOHNSTON)		
TT1708	*ara-9 argI537 leuD798 fol-101 proAB47*/ F'71–56–14 *pro*+ *lacI*^{q1}(*I*+ *Z*+) *lacI475*::Tn*10*	(C. F. BECK)		
TT1769	*pyrB692*::Tn*10 argI537 his-9533*	T	TT1704	TT1768
TT1770	*zee-1*::Tn*10 his*+ *zee-2*::Tn*10*	T	TT580	TT513
TT3227	*his-9533 strA1 recA1*	C	TT1704	TR2246
TT3606	(*hisO1242 hisDC2236*)Tn*10*(*his*+) (A duplication of this *his* region formed by recombination between *zee-2*::Tn*10* and *zee-1*::Tn*10*)	T	TT580	TT513
TT3607	*zee-1*::Tn*10* (*his*+) *zee-2*::Tn*10*/F'ts 114 *lac*+ *zzf-20*::Tn*10*	C	TT1770	TT627
TT3608	*hisIE640 proAB47 srl-201 strA1*/F'ts 114 *lac*+ *zzf-20*::(Tn*10-his*+-Tn*10*)	C	TR5128	TT3607
TT3609	*his-9533*/F'ts 114 *lac*+ *zzf-20*::(Tn*10-his*+-Tn*10*)	C	TT1704	TT3608
TT3610	*hisIE640 proAB47 strA1 recA1*/F'ts 114 *lac*+ *zzf-20*::(Tn*10-his*+-Tn*10*)	C	TR5127	TT3609
TT3611	*pyrB692*::(Tn*10-his*+-Tn*10*) *argI537 his-9533*	T	TT1768	TT1770
TT3612	*pyrB692*::Tn*10 argI537 his-9533* (Tn*10-his*+-Tn*10*; *his* operon integrated at unknown location *via* Tn*10*-directed transposition)	T	TT1768	TT1770
TT3613	*his-9533 strA1*	C	TT1704	TR2246
TT3614	*his-9533*/F'71–56–14 *pro*+ *lacI*^{q1} (*I*+ *Z*+) *lacI475*::Tn*10*	C	TT1704	TT1708
TT3615	*his-9533*/F'71–56–14 *pro*+ *lacI*^{q1} (*I*+ *Z*+) *lacI475*::(Tn*10-his*+-Tn*10*)	T	TT3614	TT1770
TT3616	*his-9533*/F'71–56–14 *pro*+ *lacI*^{q1} (*I*+ *Z*+) *lacI475*::(Tn*10-his*+-Tn*10*)	T	TT3614	TT1770
TT3617	*his-9533*/F'71–56–14 *pro*+ *lacI*^{q1} (*I*+ *Z*+) *lacI475*::(Tn*10-his*+-Tn*10*)	T	TT3614	TT1770
TT3618	*hisIE640 proAB47 strA1 recA1*/F'71–56–14 *pro*+ *lacI*^{q1} (*I*+ *Z*+) *lacI475*::(Tn*10-his*+-Tn*10*)	C	TR5127	TT3615
TT3619	*hisIE640 proAB47 strA1 recA1*/F'71–56–14 *pro*+ *lacI*^{q1} (*I*+ *Z*+) *lacI475*::(Tn*10-his*+-Tn*10*)	C	TR5127	TT3616
TT3620	*hisIE640 proAB47 strA1 recA1*/F'71–56–14 *pro*+ *lacI*^{q1} (*I*+ *Z*+) *lacI475*::(Tn*10-his*+-Tn*10*)	C	TR5127	TT3617
TR2246	HfrB2 *recA1 strA1 metA22*	(J. WYCHE)		

Strain	Genotype	Method	Recipient	Donor
TR5050	*hisIE640 proAB47 srl-201*	(J. ROTH)		
TR5127	*hisIE640 proAB47 strA1 recA1*	C	TR5050	TR2246
TR5128	*hisIE640 proAB47 strA1 srl-201*	C	TR5050	TR2246
NK337	*leu-414 sulI+* (P22 *c2ts29* 12^{-}_{amN11} 13^{-}_{amH101} *int3 TC10*)	(KLECKNER, *et al.* 1975)		
NK397	*zee-1*::Tn*10*	(N. KLECKNER)		

All strains are derivatives of *Salmonella typhimurium* LT2. Strain numbers and full genotypes are listed. The column headed "Method" refers to the genetic manipulation involved in the isolation or construction of the strain. "H" indicates *rec*-independent transposition (KLECKNER *et al.* 1975), "T" indicates P22-mediated transduction and "C" indicates bacterial conjugation. Where the method of formation and parent strains are not given, a reference or source for the strain is entered.

The ability to transpose interesting genes to predetermined locations opens several lines of inquiry, including the possibility of bringing unrelated genes close together, with the further purpose of forming operon fusions that can be useful to studies of gene regulation (reviewed by FRANKLIN 1978 and BASSFORD *et al.* 1978). The structure Tn*10-his*-Tn*10* provides a simple means of transposing the *his* operon to preselected sites, where it will be integrated in a predetermined orientation with respect to neighboring regions of the chromosome. This method should permit directed transposition of any bacterial genes.

Although the work described here has been done in Salmonella with the *his* operon and Tn*10*, similar results could reasonably be expected with other bacterial systems and other transposable elements. L. GUARENTE and T. SILHAVY (in preparation) have recently used a Tn*5-malK*-Tn*5* donor to mediate transposition of the *malK* gene in *E. coli*. Gene transposition directed by transposon homology also bears some similarities to the methods of FAELEN and TOUSSAINT (1976) and CASADABAN (1976), which rely on phage Mu homology to transpose genes in *E. coli*.

MATERIALS AND METHODS

Bacterial strains: Table 1 lists the numerical designations, full genotypes and sources or derivations of the strains used for this study. All strains are derived from *Salmonella typhimurium* LT2.

Media: Difco nutrient broth (NB, 8 g/l), with NaCl added (5 gl/), was used as rich medium. The E medium of VOGEL and BONNER (1956), containing 2% glucose, was used as minimal medium. To select for growth on lactose as sole carbon source, the NCE (modified E) medium described by BERKOWITZ *et al.* (1968) was used, supplemented with 0.2% lactose, final concentration. Medium was solidified as required by the addition of Difco agar, 1.5% final concentration.

The following additives were included in media as required (final concentrations are given): tetracycline (Sigma Chemical Co.; 25 μg/ml in rich medium or 10 μg/ml in minimal medium), streptomycin sulfate (Sigma Chemical Co.; 2 mg/ml), histidine (0.1 mM), proline (2 mM), arginine (0.6 mM), uracil (0.1 mM) and leucine (0.3 mM).

Lactose tetrazolium indicator plates (Lac TTC, LEDERBERG 1948) contained, per liter, 23 g Difco nutrient agar, 1 g NaCl, 50 mg Difco Bacto-TTC and 50 ml of a filter-sterilized 20% lactose solution, added after autoclaving the other ingredients.

Transductional methods: Bacteriophage P22 containing the mutations HT105/1 (SCHMIEGER 1971), which causes an increased frequency of generalized transduction, and *int-201* (ANDERSON and ROTH 1978), which prevents stable lysogen formation, was used for all transductions. Phage were grown on donor strains as described by SCOTT, ROTH and ARTZ (1975). In most crosses, phage and bacteria were mixed directly on selective media. When tetracycline resistance (Tet^R) was selected, phage and bacteria were mixed and preincubated for 30 min in a nonselective liquid medium before plating on tetracycline-containing medium. Transductant clones were purified and made phage free by streaking alternately on rich and selective media.

Conjugational methods: Plasmid donors were grown selectively to full density in liquid medium and then diluted 1:50 into nutrient broth. These cultures were then grown with shaking for two to four hours, generally at 37°. Strains containing F'ts114 *lac*+ (JACOB, BRENNER and CUZIN 1963; CUZIN and JACOB 1967) were grown at 30°, and subsequent matings were also performed at 30°. Recipients were grown in NB. Donor and recipient were mated on plates, either by cross-streaking or by mixing drops of each and streaking from that spot. As controls, donor and recipient were plated alone. When streptomycin resistance was selected, the best results were obtained when donor and recipient were first mated nonselectively on an NB plate for several hours and then replica printed to selective medium.

Determination of genetic instability: To determine the stability of the His+ phenotype of relevant strains, tubes containing 2 ml of liquid NB were inoculated with single colonies picked from plates containing no histidine. These cultures were grown to full density, with shaking, usually at 37°; 0.1 ml of a 10^{-6} fold dilution was then spread on an E plate containing 1/20 the normal histidine concentration. On these plates, His- bacteria form easily recognizable small pale colonies with a characteristic peak in the center. Presumptive His- segregants were picked from low-histidine plates, transferred to fully supplemented E plates and replica printed to determine the full phenotype. To determine the stability of the His+ phenotype in stains carrying F'ts114 *lac*+, this same procedure was followed, but all operations were performed at 30°. The stability of the F'ts114 *lac*+ plasmid itself was determined by growing plasmid-containing strains selectively at 30°, diluting 10^{-3}-fold into nutrient broth, growing them to full density at 42° and then plating for single colonies either on Lac TTC plates or on low-histidine plates, when the *his* genes were thought to be integrated into the plasmid.

Genetic nomenclature: We have generally followed the nomenclature conventions for insertion mutations suggested by CAMPBELL *et al.* (1977) and by CHUMLEY, MENZEL and ROTH (1979). However, the genetic rearrangements described in this paper present some new nomenclature problems. For example, the Tn*10* element that joins the tandem *his* copies in the duplication strain shown in Figure 1b is a "hybrid" consisting of the "left" end of the insertion, *zee-2*::Tn*10*, and the "right" end of the insertion, *zee-1*::Tn*10*. (*zee-1*::Tn*10* and *zee-2*::Tn*10* are insertions near the *his* operon that do not affect any known gene; for a discussion of this type of nomenclature, see CHUMLEY, MENZEL and ROTH 1979.) This join-point Tn*10* insertion should not be named either *zee-1*::Tn*10* or *zee-2*::Tn*10*, and its position cannot be related to the wild-type Salmonella chromosome in any simple way. Naming the endpoints of the duplicated segment also presents a problem, as they are unmarked and can be known only as the sites in which *zee-1*::Tn*10* and *zee-2*::Tn*10* are carried by strains that contain those insertions. For the sake of simplicity, we have chosen to represent the genotype of the strain shown in Figure 1b as *hisDC2236*-Tn*10*-*his*+, but one should be aware of the complexities of this structure, as well as possible differences from similar genetic rearrangements that may be formed in crosses involving other parental insertion mutations.

Similar problems exist in naming structures such as that in TT3611, which carries the *his* operon transposed to the *pyrB* locus. Since the *his* region has been integrated *via* Tn*10* homology into an allele of *pyrB* (*i.e.*, *pyrB692*::Tn*10*), which had previously been named in accordance with accepted practice (DEMEREC *et al.* 1966; CAMPBELL *et al.* 1977), we have preserved the numerical identity of that allele, indicating that the inserted material has become more complex. For example, *pyrB692*::Tn*10* following transposition becomes *pyrB692*::(Tn*10*-*his*+-Tn*10*). This seems a particularly suitable designation since this structure, when carried in an otherwise wild-type genetic background, must exist in equilibrium between the two states:

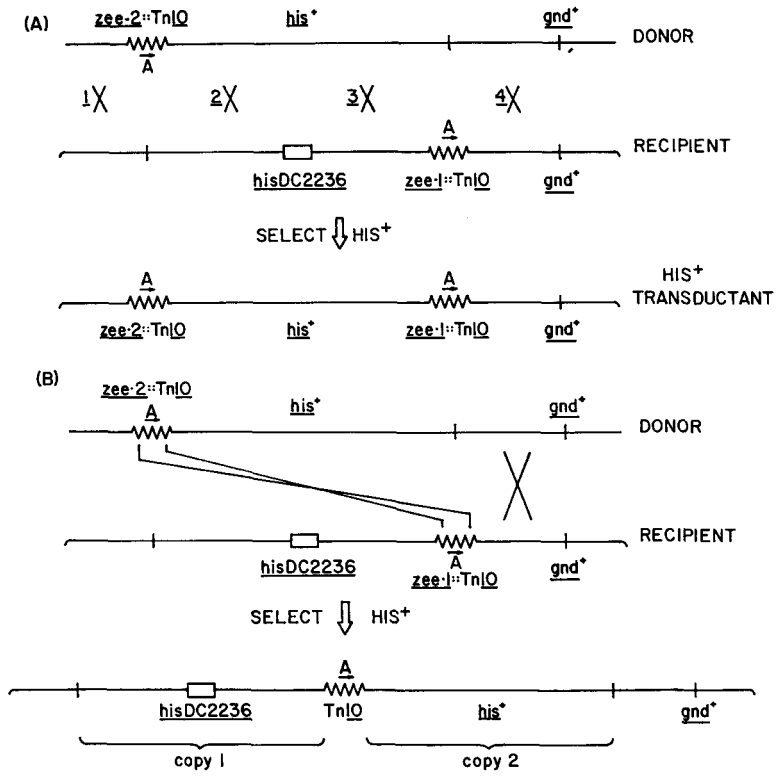

FIGURE 1.—Recombination events leading to the formation of Tn*10-his*+-Tn*10* (Figure 1a) and *his*-Tn*10-his* (Figure 1b). The donor strain is TT513 (which is *his*+ and carries *zee-2*::Tn*10*), and the recipient is strain TT580 (*hisDC2236 zee-1*::Tn*10*). The Tn*10* insertions in the donor and recipient have the same orientation (orientation "A"), as determined by Tn*10*-directed Hfr formation (CHUMLEY, MENZEL and ROTH 1979). His+ transductants are selected on E minimal medium, as described in MATERIALS AND METHODS. In Figure 1a, recombination events one and three yield a His+ transductant that contains both *zee-2*::Tn*10* and *zee-1*::Tn*10*. Events one and four, two and three, or two and four will produce His+ transductants that contain either of the Tn*10* insertions alone or neither of the Tn*10* insertions. In Figure 1b, unequal recombination between Tn*10* elements yields His+ recombinants that contain two copies of the *his* region joined by a single Tn*10* element.

pyrB692::(Tn*10-his*+-Tn*10*)↔*pyrB692*::Tn*10* + circular fragment.

As discussed in RESULTS, the transposed material in such rearrangements will be integrated at the target Tn*10* insertion in an orientation with respect to flanking regions determined by the orientation of the three Tn*10* insertions involved in catalyzing the transposition. In some connections it will be important to know this orientation, and we would suggest that where the transposition involves integration into the bacterial chromosome or into bacterial genes carried on a plasmid, the transposed genes should be listed in the order in which they would actually be encountered tracing along the chromosome in a clockwise direction [*i.e.*, *lacI475*::(Tn*10-hisEIFAHBCDGO*-Tn*10*)]. Tn*10* insertions can be assigned either an "A" or "B" orientation (CHUMLEY, MENZEL and ROTH 1979); when required, this orientation could be indicated in parentheses following the Tn*10* designation [*i.e.*, *hisC8556*::Tn*10*(B)].

The nomenclature for the plasmid carried by TT1708 (F'71-56-14 *pro*+ $i^{91}|\underline{i+\ z+}|$) is that

of MÜLLER-HILL and KANIA (1974) and BRAKE *et al.* (1978), who have described this plasmid and characterized the fused *lacI-lacZ* protein.

RESULTS

*Construction of Tn*10-his-*Tn*10 *and* his-*Tn*10-his*:* To construct a strain in which the *his* region is closely flanked by direct repeats of Tn*10*, P22 phage was grown on TT513, which is *his*$^+$ and carries a *hisO*-side Tn*10* insertion in the "A" orientation (CHUMLEY, MENZEL and ROTH 1979). This strain was then used as a transductional donor in a cross with TT580, selecting His$^+$ recombinants. TT580 contains a *hisDC* deletion and a *hisE*-side Tn*10* insertion, also in the "A" orientation. Figure 1a diagrams this cross; the recombination events that must occur to yield a Tn*10-his*-Tn*10* transductant are events 1 and 3. Other recombination events could occur to yield His$^+$ transductants that inherit either of the Tn*10* insertions alone or neither Tn*10* insertion. We expected to identify Tn*10-his*-Tn*10* transductants as His$^+$ Tet^R recombinants that were unstable for the His$^+$ phenotype. The results of the transduction are reported in Table 2. The stability of His$^+$ in His$^+$ Tet^R recombinants was determined as described in MATERIALS AND METHODS. The frequency of His$^-$ segregants was about 0.5% in all unstable isolates, while no His$^-$ segregants were detected from other recombinants tested (a frequency of less than 0.05%).

The His$^-$ segregants from unstable isolates were examined for Tet^R by replica printing to rich medium containing tetracycline. We discovered that the unstable His$^+$ Tet^R isolates indicated in Table 2 fell into two classes. Some of the unstable isolates (Class 1) yielded only His$^-$ Tet^R segregants. The other unstable isolates (Class 2) yielded mostly His$^-$ Tet^s segregants along with some (10% or fewer) His$^-$ Tet^R segregants. The segregation properties of Class 1 isolates are consistent with expectations for strains carrying Tn*10-his*-Tn*10*, as shown in Figure 2. The behavior of Class 2 isolates (giving rise primarily to His$^-$ Tet^S segregants) suggested the formation of some other unstable *his*$^+$ structure.

To further characterize Class 1 and Class 2 isolates, we mapped the *his*$^-$ defect

TABLE 2

*Results of transduction experiment yielding Tn*10-his-*Tn*10 *and* his-*Tn*10-his *recombinants*

His$^+$ donor	His$^-$ recipient	Fraction of His$^+$ recombinants that are Tet^R (a)	Fraction of tested His$^+$ recombinants that are unstably His$^+$ (b)	Unstable recombinants yielding His$^-$ Tet^R segregants (c)	Unstable recombinants yielding His$^-$ Tet^S segregants (d)
TT513 (*zee-2*::Tn*10*)	TT580 (*hisDC2236 zee-1*::Tn*10*)	43/50	7/24	3/7	4/7

His$^+$ recombinants were selected as described in MATERIALS AND METHODS. (a) Randomly chosen His$^+$ transductants were checked for Tet^R by replica printing. (b) His$^+$ Tet^R transductants were examined for instability of His$^+$, as described in MATERIALS AND METHODS. (c) These isolates yielded His$^-$ segregants, all of which were Tet^R. (d) These isolates yielded His$^-$ segregants, most of which were Tet^S.

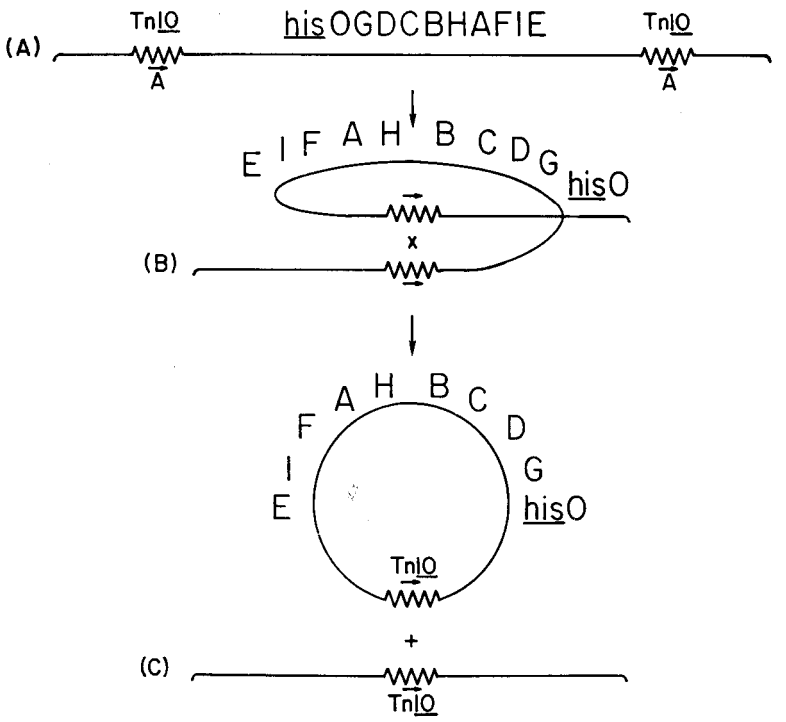

FIGURE 2.—Recombination between Tn*10* sequences flanking the histidine operon in Tn*10*-*his*+-Tn*10* results in the deletion of the entire *his* region and the production of a circular chromosome fragment containing the *his* operon and a single Tn*10* element.

in the segregants, using them as recipients in transductions, and selecting His+ recombinants with various *his*⁻ point mutants as donors. All the *his*⁻ segregants arising from Class 1 isolates mapped as deletions of the entire *his* operon (five were tested). We concluded that Class 1 transductants (for example, TT1770) have the structure Tn*10*-*his*-Tn*10*. Further deletion-mapping crosses confirmed the presence of the two Tn*10* insertions (*zee-1*::Tn*10* and *zee-2*::Tn*10*) in Class 1 isolates. (Putative Tn*10*-*his*-Tn*10* *isolates* were used as *his*+ donors in transductions with recipients containing *his* deletions extending varying distances beyond either end of the *his* operon; the frequency of Tet^R was scored in the crosses.) All the *his*⁻ segregants arising from Class 2 isolates mapped as deletions of the *hisD* and *hisC* genes (10 were tested). These ten segregants carry a deletion mutation indistinguishable from *hisDC2236*. This suggests that these transductants have two copies of *his* region (*hisDC2236* and *his*+) with an intervening Tn*10* element. Strain TT3606 is an example of this duplication class, which arises from the unequal crossing over events shown in Figure 1b.

The relatively rare His⁻ Tet^R segregants obtained from strains carrying *hisDC2236*-Tn*10*-*his*+ are somewhat surprising because recombination events between the *his* regions producing His⁻ haploid segregants should result only in a Tet^S phenotype. Two explanations of the Tet^R segregant class suggest them-

selves. First, these segregants may still carry the *his* duplication, with *hisDC2236* in both copies (*hisDC2236*-Tn*10*-*hisDC2236*). Such homozygosity for *hisDC2236* could arise through any of several types of recombination events. A second possibility is that the His$^-$ Tet^R segregants might carry new *his* deletions generated by the joint-point Tn*10* insertion (CHAN and BOTSTEIN 1972; KLECKNER, REICHARDT and BOTSTEIN 1979). This would seem likely only if the frequency of deletion generation by the "hybrid" join-point Tn*10* insertion were enhanced relative to a normal Tn*10* insertion (see Section F in MATERIALS AND METHODS).

"Levitation" of the his *operon:* The recombination events predicted in Figure 2 suggest that the homologous Tn*10* sequences flanking the *his* region in Tn*10*-*his*-Tn*10* should provide a means for transposing the *his* genes to the site of some other Tn*10* insertion, where they can be integrated by a single crossover between the Tn*10* element in the circular fragment represented in Figure 2 and the target Tn*10* element. The integration process would essentially be the reverse of the events shown in Figure 2. We have used this scheme to isolate a strain in which the *his* operon has undergone translocation from the chromosome to the F′ plasmid, F′ts114 *lac*$^+$ (JACOB, BRENNER and CUZIN 1963; CUZIN and JACOB 1967). We have designated this process gene levitation. (This transposition can also occur without a circular intermediate; see SCHMID and ROTH 1980.)

F′ts114 *lac*$^+$, carrying the Tn*10* insertion *zzf-20*::Tn*10*, was transferred from TT627 into TT1770, selecting Lac$^+$ at 30°. (Wild-type *S. typhimurium* has no genes for lactose utilization.) The resulting strain, TT3607, contains three Tn*10* insertions and has the genotype, *zee-1*::Tn*10* (*his*$^+$) *zee-2*::Tn*10*/F′ts114 *lac*$^+$ *zzf-20*::Tn*10*. To determine whether any individuals in the culture of TT3607 carry the *his* operon levitated into F′ts114*lac*$^+$, this strain was mated with a *his*$^-$ Sm^R recipient (TR5128), selecting for transfer of either Lac$^+$ or His$^+$, as described in MATERIALS AND METHODS. Under conditions where 2000–3000 Lac$^+$ transconjugants were obtained, only seven His$^+$ transconjugants appeared. These His$^+$ isolates were purified by streaking alternately on rich and selective media, and they were then examined for other genetic characteristics. All seven isolates were His$^+$ Tet^R, but six proved to be Lac$^-$, while one was Lac$^+$. In subsequent conjugation with TT1704 (which carries a deletion removing the entire *his* region), the six His$^+$ Tet^R Lac$^-$ isolates failed to transfer any markers, while the single His$^+$ Tet^R Lac$^+$ isolate transferred Tet^R Lac$^+$ simultaneously upon selection of transfer of His$^+$. This single His$^+$ Tet^R Lac$^+$ isolate, TT3608, was presumed to contain an F′ts114 *lac*$^+$ plasmid carrying the complex insertion, *zzf-20*::(Tn*10*-*his*$^+$-Tn*10*).

The plasmid was then transferred from strain TT3609 into the *his*$^-$ *recA*$^-$ strain, TR5127, selecting His$^+$ transconjugants. (TT3609 was chosen as a representative transconjugant from this mating.) The six nonfertile His$^+$ Tet^R Lac$^-$ isolates may have arisen through standard recombination events following transfer of the *his* region from TT3607 due to Tn*10*-directed Hfr formation (CHUMLEY, MENZEL and ROTH 1979).

To learn more about the structure of the putative F′ *his* plasmid, the stability of the *his*$^+$ marker was determined following growth at 30° or 42°; both *rec*$^+$

(TT3608) and *rec*⁻ (TT3610) strains were tested. The results of this experiment are shown in Table 3. All His⁻ derivatives isolated at 30° from the *rec*⁺ strain, TT3608, remained Lac⁺ and Tet^R (26 were tested). These segregants presumably arose by excision of Tn*10-his*-Tn*10* from the F′ *lac* Tn*10* plasmid. All His⁻ derivatives isolated from TT3608 at 42° were His⁻ Lac⁻ Tet^S and seemed to have lost the entire plasmid. This behavior is expected since any genetic markers carried on the F′ts114 *lac*⁺ plasmid should be lost with very high frequency during growth at 42°, owing to the inability of the plasmid to replicate at high temperature. All segregants from the *rec*⁻ strain (TT3610) were also His⁻ Lac⁻ Tet^S and had lost the entire plasmid; apparently the *rec*⁻ mutation prevents excision of Tn*10-his*⁺-Tn*10*.

These results are strong evidence that the structure of the plasmid carried by TT3608, 3609 and 3610 is F′ts114 *lac*⁺ *zzf-20*::(Tn*10-his*⁺-Tn*10*). The difference in the frequency and properties of His⁻ segregants derived from TT3608 (*rec*⁺) and TT3610 (*recA*⁻) indicates that *recA* function is essential for the Tn*10* × Tn*10* recombination events that give rise to His⁻ segregants from the structure Tn*10-his*-Tn*10*.

Strains carrying Tn*10-his*-Tn*10* in the chromosome yield His⁻ segregants at a frequency of about 0.5% (reported above). As seen in Table 3, the same Tn*10-his*-Tn*10* structure is 30-fold less stable on an F′ plasmid than in the chromosome. This pattern has held true for other Tn*10*-directed transpositions of *his* reported below. The significance of this observation is not immediately apparent. Intramolecular Tn*10* × Tn*10* exchanges might occur more frequently in an F′ plasmid, or perhaps the plasmid copy number enhances the frequency of unequal Tn*10* × Tn*10* exchanges of a type resulting in one recombinant plasmid with the complex insertion *zzf-20*::(Tn*10-his*⁺-Tn*10-his*⁺-Tn*10*) and another (*his*⁻) plasmid with the simple *zzf-20*::Tn*10* insertion.

Transposition of the his operon mediated by generalized transduction: When phage P22 is grown on a strain containing Tn*10-his*-Tn*10* and used as a donor of *his*⁺ in generalized transduction, recombinants can arise in which the *his* genes have been integrated at the site of a Tn*10* insertion resident in the recipient

TABLE 3

The effect of incubation temperature and recA *function on the stability of the His⁺ phenotype in strains carrying the* his *genes on F′ts114* lac.

Strain and genotype		Frequency of His⁻ segregants following growth in NB at 30° or 42°	
TT3608:	*hisIE640 proAB47 strA1 rec*⁺/F′ts114 *lac*⁺ *zzf-20*::(Tn*10-his*⁺-Tn*10*)	15% (3700[a])	99% (250)
TT3610:	*hisIE640 proAB47 strA1 recA1*/F′ts114 *lac*⁺ *zzf-20*::(Tn*10-his*⁺-Tn*10*)	0.1% (3400)	75% (200)

The frequency of His⁻ segregants was determined as described in MATERIALS AND METHODS.
(a) The numbers in parentheses indicate the total number of colonies examined on low-histidine plates.

genome. Using this transductional method, the *his* operon has been transposed to the site of a *pyrB*::Tn*10* insertion, producing the complex insertion, *pyrB*::(Tn*10*-*his*$^+$-Tn*10*).

Strain TT1769 contains the large deletion *his-9533*, which removes the entire *his* operon and so much of the neighboring chromosomal regions that it cannot be repaired in P22-transduction by a single *his*$^+$ transduced fragment (H. M. JOHNSTON, personal communication; F. CHUMLEY, unpublished). Strain TT1769 also contains the linked mutations *pyrB692*::Tn*10* and *argI537*. Transducing phage was grown on donor strain TT1770 [*zee-1*::Tn*10* (*his*$^+$) *zee-2*::Tn*10*] and mixed with recipient strain TT1769, selecting His$^+$ transductants. His$^+$ recombinants, all of which remained Pyr$^-$ Arg$^-$ Tet^R, were recovered at a frequency of 2.4×10^{-7} per plaque-forming unit. This frequency is 10^2- to 10^3-fold less than the frequency of transduction for a simple *his*$^-$ point mutation. When LT2 was used as a *his*$^+$ donor, no His$^+$ recombinants were detected (a frequency of less than 7×10^{-10} His$^+$ transductants per plaque-forming unit). Ten independent His$^+$ transductants were chosen from the cross with TT1770 as donor, and these transductants were further analyzed for the stability and map position of the *his* genes.

These His$^+$ transductants fell into two classes. Eight of the ten transductants (Class 1) segregated His$^-$ individuals at a frequency of about 0.5%, while the two remaining isolates (Class 2) did not segregate His$^-$ individuals at a detectable rate (less than 0.05%). In subsequent transduction crosses, the *his*$^+$ marker in Class 1 isolates behaved as an allele of the *pyrB* locus. That is, all recombinants made *pyr*$^+$ by transduction became simultaneously *His*$^-$ and Tet^S (indicating that the *pyrB692*::(Tn*10*-*his*$^+$-Tn*10*) insertion was displaced). Furthermore, 60% of argI$^+$ transductants also became Pyr$^+$ His$^-$ Tet^S, while 40% remained Pyr$^-$ His$^+$ Tet^R. This distribution reflects the normal *argI-pyrB* transductional linkage (SYVANEN and ROTH 1972). Class 2 isolates showed no such association of *pyrB* and *his*. It is apparent, then, that Class 1 isolates (such as TT3611) carry the insertion *pyrB692*::(Tn*10*-*his*$^+$-Tn*10*), while Class 2 isolates (such as TT3612) carry the *his* genes at some other location.

Although we have not investigated Class 2 isolates further, they probably contain the *his* genes integrated in residual Tn*10* homology known to be associated with the *his-9533* deletion in the recipient strain TT1769 (F. CHUMLEY, unpublished). This deletion was generated by *zee-1*::Tn*10* (H. M. JOHNSTON, personal communication; H. M. JOHNSTON and J. R. ROTH 1979). In the absence of any Tn*10* insertion resident in the chromosome, a *rec*$^+$ strain containing *his-9533* (TT3613) can still undergo *his*$^+$ transduction with TT1770 as donor at a frequency reduced about ten-fold to 2.6×10^{-8} transductants per plague-forming unit. If *recA* function is eliminated (using TT3227 as recipient), the frequency falls to an undetectable level, less than 1×10^{-10}. It seems possible that the structure Tn*10*-*his*-Tn*10* might give rise to one or more kinds of new transposable elements that include the *his* operon and are able to insert into the chromosome through a *rec*-independent process. Efforts to detect such illegitimate transposition of *his* have thus far proven unsuccessful (F. CHUMLEY, unpublished).

Translocation of the his *operon to an F′ plasmid by generalized transduction:* Relying on the transductional method for integrating the *his* genes at novel sites, we have placed the *his* operon on an F′*lac* plasmid, where it was integrated into a *lacI*::Tn*10* insertion. The recipient (TT3614) contained the large deletion his-*9533* and an F′*lac* plasmid with a deletion fusing the *lacI* and *lacZ* genes (shown in Figure 3). This plasmid was derived from F′128 *pro*$^+$ *argF*$^+$ *lac*$^+$ by MÜLLER-HILL and KANIA (1974), and its deletion was characterized by BRAKE *et al.* (1978). A *lacI*::Tn*10* mutation was introduced into this plasmid by C. F. BECK (personal communication). The *lac* genes on this plasmid are not expressed because of the presence of the *lacI475*::Tn*10* insertion; this insertion has two effects. First the Tn*10* insertion prevents transcription of *lac* by acting as an absolute polar block on transcription initiating from the *lacI*^{Q1} promoter; since the normal *lac* promoter is deleted, the polar block of the *lacI*::Tn*10* prevents *lacZ* transcription. Second, the Tn*10* insertion cleaves the fused *lacI-lacZ* gene, removing the translation-initiation site (in *lacI*) from the remaining *lacZ* coding sequences. By using this Tn*10* insertion as a target for Tn*10*-directed translocation of *his*, it has been possible to integrate the *his* operon near the unexpressed *lacZ*, *Y* and *A* genes.

A recipient strain carrying the F′ plasmid described above (TT3614) was mixed with transducing phage grown on a donor strain carrying the structure Tn*10*-*his*-Tn*10* (TT1770); His$^+$ transductants were recovered. Each of 30 of these His$^+$ transductants was mated with a *pro*$^-$ *his*$^-$ *rec*$^-$ recipient (TR5127), selecting transfer of Pro$^+$. All 30 transductants transferred Pro$^+$ efficiently (and Tet^R as well); 28 were discovered to have simultaneously transferred His$^+$, which was unselected in the conjugation. This confirmed that most of these

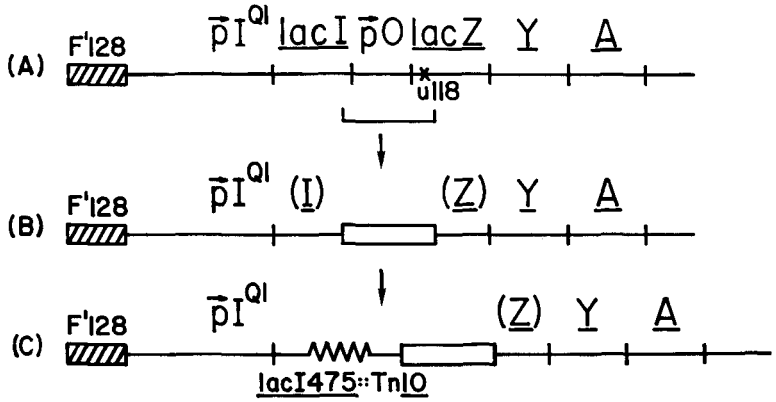

FIGURE 3.—Derivation and structure of the F′71–56–14 plasmid carrying *lacI475*::Tn*10*. Beginning with the F′ 128 *pro lac* plasmid shown in Figure 3a, MÜLLER-HILL and KANIA (1974) isolated a derivative (Figure 3b) in which the *lacI* and *lacZ* genes are fused, producing a single polypeptide chain with both *lacI* and *lacZ* activities. C. BECK (personal communication) introduced the *lacI475*::Tn*10* insertion into the remaining *lacI* sequences, generating the plasmid represented in Figure 3c. The *lacI475*::Tn*10* insertion prevents the expression of the *lac* coding sequences remaining on the plasmid.

transductants had inherited *his*+ at a site on the plasmid. The stability of the His+ phenotype was then examined in *rec*+ and *rec*- isolates. In the *rec*+ background (TT3615–TT3618), His- segregants that remained Tet^R were found at a frequency of about 10%, while in a *recA*- background (TT3618–TT3620) this frequency was decreased to less than 0.1%. Based on this behavior and on considerations presented below, we conclude that the F′ plasmid containing the *his* transposition was formed as diagrammed in Figure 4.

It should be noted that, in all the foregoing transpositions and translocations, the orientation of the integrated *his* operon with respect to neighboring regions is determined by the orientation of the target Tn*10* insertion. If the orientation of the *lacI*::Tn*10* insertion in Figure 4 were reversed, the *his* genes would be integrated in the opposite order with respect to the *lac* genes. On the plasmid carried by strain TT3615, transcription initiating in *hisO* must proceed towards the *lac* genes, because it has been relatively easy to isolate a large number of derivatives of this strain in which the *lac* genes are expressed under the control of the *his* operon (F. CHUMLEY and J. ROTH, in preparation).

DISCUSSION

Homologous recombination between Tn*10* elements inserted at various points in the bacterial chromosome or in bacterial plasmids can result in chromosome rearrangements with useful genetic properties. We have used Tn*10* homology (1) to promote the formation of deletions and duplications with predetermined endpoints and (2) to direct the *rec*-dependent transposition of bacterial genes to sites far from their normal location. Tn*10* insertions in or near almost any gene of interest can be isolated with ease. It should be relatively straightforward

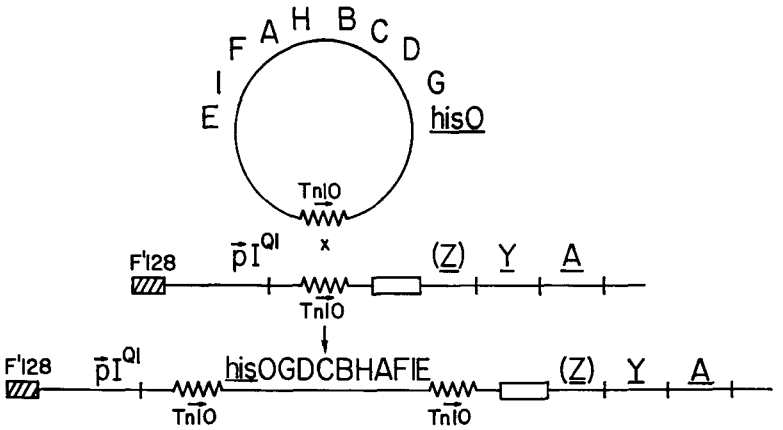

FIGURE 4.—Translocation of the *his* operon to the F′ 71–56–14 plasmid carrying *lacI475*::Tn*10*. Using Tn*10-his*+-Tn*10* (strain TT1770) as a donor of His+ in P22-mediated transduction, a circular chromosome fragment containing the *his* operon and a single Tn*10* element may form (see text). Recombination between the Tn*10* sequence in this circle and *lacI475*::Tn*10* integrates the *his* genes into the plasmid near the unexpressed *lacZ*, *Y* and *A* genes. The orientation of the integrated *his* genes is determined by the orientation of the Tn*10* insertions involved.

to extend these methods to bacterial genetic systems and transposable elements other than those we have used.

In crosses designed to yield the genetic structure, Tn*10*-*his*-Tn*10*, another structure containing a *his* duplication, *his*-Tn*10*-*his*, is formed with roughly equal frequency, as described above. This probably reflects the lengths of the regions available for recombination. The *his* region and the Tn*10* elements both represent large stretches of homology, so that the types of exchanges shown in Figure 1a and 1b are equally likely. The facility with which duplications of the type, *his*-Tn*10*-*his*, can be identified suggests a means of complementation testing for bacterial gene clusters. Using the appropriate Tn*10* insertions in a transductional cross shown in Figure 1b, it should be possible to duplicate any chromosomal segment. Working in this laboratory, L. Bossi (in preparation) has successfully applied chromosomal duplications to the dominance testing of frameshift suppressor mutations.

The directed transposition techniques described, either involving gene levitation or transposition mediated by transduction, should be most useful for bringing genes of interest into close proximity for the purpose of isolating operon fusions (Franklin 1978; Bassford *et al.* 1978). As demonstrated in the accompanying paper (Schmid and Roth 1980), Tn*10*-directed transpositions of these types can proceed *via* a circular fragment containing the *his* genes and a single Tn*10* element. Certainly, the structure of the region integrated at the target site (Tn*10*-*his*-Tn*10*) is consistent with such a mechanism. The *lacI*::Tn*10* insertion carried in the compound *lacI*-*lacZ* gene on the F′ plasmid described may provide an ideal and generally useful target for Tn*10*-directed transposition for the purpose of fusing the *lac* genes to interesting foreign control elements.

LITERATURE CITED

Anderson, R. P. and J. R. Roth, 1978 Tandem chromosomal duplications in *Salmonella typhimurium*: fusion of histidine genes to novel promoters. J. Mol. Biol. **119**: 147–166.

Bassford, P., J. Beckwith, M. Berman, E. Brickman, M. Casadaban, L. Guarente, I. Saint-Girons, A. Sarthy, M. Schwartz, H. Shuman and T. Silhavy, 1978 Genetic fusions of the *lac* operon: a new approach to the study of biological processes. pp. 245–261. In: *The Operon*. Edited by J. H. Miller and W. S. Reznikoff. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

Berkowitz, D., J. M. Hushon, H. J. Whitfield, Jr., J. Roth and B. N. Ames, 1968 Procedure for identifying nonsense mutations. J. Bacteriol **96**: 215–220.

Brake, A. J., A. V. Fowler, I. Zabin, J. Kania and B. Müller-Hill, 1978 Beta-galactosidase chimeras: Primary structure of a *lac* repressor Beta-galactosidase protein. Proc. Natl. Acad. Sci. U.S. **75**: 4824–4827.

Campbell, A., D. Berg, E. Lederberg, P. Starlinger, D. Botstein, R. Novick and W. Szybalski, 1977 Nomenclature of transposable elements in prokaryotes. pp. 15–22. In: *DNA Insertion Elements, Plasmids, and Episomes*. Edited by A. I. Bukhari, J. A. Shapiro, and S. L. Adhya. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

Casadaban, M. J., 1976 Transposition and fusion of the *lac* genes to selected promoters in *Escherichia coli* using bacteriophage lambda and Mu. J. Mol. Biol. **104**: 541–555.

CASADABAN, M. J., T. J. SILHAVY, M. L. BERMAN, H. A. SHUMAN, A. V. SARTHY and J. R. BECKWITH, 1977 Construction and use of gene fusions directed by bacteriophage Mu insertions. pp. 531–535. In: *DNA Insertion Elements, Plasmids, and Episomes.* Edited by A. I. BUKHARI, J. A. SHIPIRO and S. L. ADHYA. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

CHAN, R. K. and D. BOTSTEIN, 1972 Genetics of bacteriophage P22 I. Isolation of prophage deletions which affect immunity to superinfection. Virology **49:** 257–268.

CHUMLEY, F., R. MENZEL and J. R. ROTH, 1979 Hfr formation directed by Tn*10*. Genetics **91:** 639–655.

CUZIN, F. and F. JACOB, 1967 Mutations de l'Episome F d'*Escherichia coli* K12 II. Mutations à replication thermosensible. Ann. Inst. Past. **112:** 397–418.

DEMEREC, M., E. ADELBERG, A. CLARK and P. HARTMAN, 1966 A proposal for a uniform nomenclature in bacterial genetics. Genetics **54:** 61–76.

FAELEN, M. and A. TOUSSAINT, 1976 Bacteriophage Mu-1: a tool to transpose and localize bacterial genes. J. Mol. Biol. **104:** 525–539.

FAELEN, M., A. TOUSSAINT, M. VAN MONTAGU, S. VAN DEN ELSACKER, G. ENGLER and J. SCHELL, 1977 *In vivo* Genetic Engineering: The Mu-mediated transposition of chromosomal DNA segments onto transmissible plasmids. pp. 521–530. In: *DNA Insertion Elements, Plasmids, and Episomes.* Edited by A. I. BUKHARI, J. A. SHAPIRO and S. L. ADYHA. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

FRANKLIN, N. C., 1978 Genetic fusions for operon analysis. Ann. Rev. Gen. **12:** 193–221.

JACOB, F., S. BRENNER and F. CUZIN, 1963 On the regulation of DNA replication in bacteria. Cold Spring Harbor Symp. Quant. Biol. **28:** 329–348.

JOHNSTON, H. M. and J. R. ROTH, 1979 Histidine mutants requiring adenine: selection of mutants with reduced *hisG* expression in *Salmonella typhimurium*. Genetics **92:** 1–15.

KLECKNER, N., R. K. CHAN, B.-K. TYE and D. BOTSTEIN, 1975 Mutagenesis by insertion of a drug resistance element carrying an inverted repetition. J. Mol. Biol. **97:** 561–575.

KLECKNER, N., K. REICHARDT and D. BOTSTEIN, 1979 Inversions and deletions of the *Salmonella* chromosome generated by the translocatable tetracycline resistance element Tn*10*. J. Mol. Biol. **127:** 89–115.

KLECKNER, N., J. ROTH and D. BOTSTEIN, 1977 Genetic Engineering *in vivo* Using Translocatable Drug-resistance Elements. New Methods in Bacterial Genetics. J. Mol. Biol. **116:** 126–159.

LEDERBEG, J., 1948 Detection of fermentative mutants with tetrazolium. J. Bacteriol. **56:** 695.

MÜLLER-HILL, B. and J. KANIA, 1974 *Lac* repressor can be fused to Beta-galactosidase. Nature **249:** 561–563.

SCHMID, M. and J. R. ROTH, 1980 Circularization of transduced fragments: a mechanism for adding segments to the bacterial chromosome. Genetics **94:** 15–29.

SCHMIEGER, H., 1971 A method for detection of phage mutants with altered transducing ability. Mol. Gen. Genet. **100:** 378–381.

SCOTT, J. F., J. R. ROTH and S. W. ARTZ, 1975 Regulation of histidine operon does not require *hisG* enzyme. Proc. Natl. Acad. Sci. U.S. **72:** 5021–5025.

SYVANEN, J. M. and J. R. ROTH, 1972 Structural genes for ornithine trans-carbamylase in *Salmonella typhimurium* and *Escherichia coli K-12*. J. Bacteriol. **110:** 66–70.

VOGEL, H. J. and D. M. BONNER, 1956 Acetylornithase of *Escherichia coli*: partial purification and some properties. J. Biol. Chem. **218:** 97–106.

Corresponding editor: H. ECHOLS