Molec. gen. Genet. 166, 217–223 (1978)

MGG

Evidence for a Common Mechanism for the Insertion of the Tn10 Transposon and for the Generation of Tn10-Stimulated Deletions

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Summary. Mutations in and near the *Salmonella typhimurium* histidine transport operon were generated by insertion of the translocatable tetracycline-resistance element Tn10. Deletion mutants affecting histidine transport genes were subsequently isolated in several of the Tn10-containing strains. Tn10 insertions in *hisJ* occurred preferentially at one site, designated site A. This same site was also the preferential endpoint of deletions originating from Tn10 insertions at two neighboring sites. Thus, Tn10 insertion and Tn10-stimulated deletion formation appear to involve a common DNA-recogition step.

Introduction

Translocatable genetic elements have assumed an important role in procaryotic genetic studies (Kleckner et al., 1977; Starlinger and Saedler, 1976). Drug-resistance elements, IS insertion sequences, and the bacteriophage Mu integrate at many sites in bacterial chromosomes (Kleckner, 1977; Starlinger and Saedler, 1976; Bukhari, 1976). The integration and excision of these translocatable genetic elements occur independently of the known general homologous-recombination enzymes of the host. Thus, these events are classified as "illegitimate" recombination events, and the mechanisms involved in the processes are unknown (Kleckner, 1977; Cohen, 1976). Most inserted elements are known to cause an increased frequency of deletion of bacterial DNA in the vicinity of the inserted element (Kleckner, 1977; Starlinger and Saedler, 1976; Reif and Saedler, 1975). A primary issue to be resolved is whether the mechanisms responsible for insertion, excision, and deletion are related.

The Tn10 transposon, which confers resistance to tetracycline (Kleckner et al., 1975), is composed of 9,300 basepairs and of inverted terminal repetitions at its ends which are 1,400 basepairs long (Kleckner, 1977). We have utilized its properties in fine-structure mapping of the histidine transport genes of *Salmonella typhimurium* (Ames et al., 1977). The Tn10 element exhibits some specificity with regard to the sites of its integration into bacterial chromosomes, as evidenced by "hot-spots" for insertion in the histidine operon of *Salmonella* (Kleckner et al., 1978a) and in the lactose operon of *E. coli* (Foster, 1977). Likewise, we show here that there is a preferred site for Tn10 insertion in a histidine transport gene, *hisJ*. Moreover, we show that this same site is also the predominant endpoint of deletions which terminate within *hisJ*, when such deletions are promoted by Tn10 elements inserted outside the *hisJ* gene. This coincident site specificity indicates that the mechanisms of Tn10 insertion and of Tn10-promoted chromosomal deletion have some aspect in common.

Materials and Methods

Strains. All bacterial strains are derivatives of *S. typhimurium* LT2 and are described in Table 1.

Growth of Bacteria. Nutrient broth and L broth have been described (Roth, 1970). Minimal medium was medium E of Vogel and Bonner (1956). Bacteria grown in liquid were aerated by vigorous shaking. The temperature was 37° except where noted otherwise.

Genetic Mapping. Mutant strains were crossed by transduction with P22 HT *int-201* phage (Ames et al., 1977).

Preparation of Tc-10 Phage. Tc-10 phage are P22 (12⁻*amN11* 13⁻*amH101 c2 ts29 int3* Tn10). They were released from the

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0026-8925/78/0166/0217/$01.40

Table 1. Strains

Strain number[a]	Genotype[b]	Strain number	Genotype[b]
NK40	*proC* (P22 *sieA6 int*$_3$)		*Tn10-promoted deletion mutants*
NK337	*leuA414 sup-19* (P22 Tc-10)		*Sites covered by deletion*
TA271	*dhuA1*	TA3079	*his-6619* (*ubiX*, *dhuA*, *hisJ*, *hisP*)
TA1852	*dhuA1 purF145*	TA3080	*his-6620* (*ubiX*, *dhuA*, *hisJ*, *hisP*)
		TA3081	*his-6621* (*ubiX*, *dhuA*, *hisJ*, *hisP*)
Tn10 insertion mutants		TA3082	*his-6622* (*ubiX*, *dhuA*, *hisJ*, *hisP*)
TA3088	*dhuA1 zei-102*::Tn10	TA3083	*his-6623* (*ubiX*, *dhuA*, *hisJ*, *hisP*)
TA3178 (2)	*dhuA1 hisJ8908*::Tn10	TA3084	*his-6624* (*ubiX*, *dhuA*, *hisJ*, *hisP*)
TA3183 (2)	*dhuA1 hisJ8911*::Tn10	TA3085	*his-6625* (*ubiX*, *dhuA*, *hisJ*, *hisP*)
TA3184 (3)	*dhuA1 hisJ8912*::Tn10	TA3099	*his-6626* (*ubiX*, *dhuA*, *hisJ*, *hisP*)
TA3185 (2)	*dhuA1 hisJ8913*::Tn10	TA3100	*his-6627* (*ubiX*, *dhuA*, *hisJ*, *hisP*)
TA3186 (2)	*dhuA1 hisJ8914*::Tn10	TA3159	*his-6628* (*ubiX*, *dhuA*, *hisJ*, *hisP*)
TA3187 (2)	*dhuA1 hisJ8915*::Tn10	TA3160	*his-6629* (*ubiX*, *dhuA*, *hisJ*, *hisP*)
TA3188 (2)	*dhuA1 hisJ8916*::Tn10	TA3161	*his-6630* (*ubiX*, *dhuA*, *hisJ*, *hisP*)
TA3189 (2)	*dhuA1 hisJ8917*::Tn10	TA3162	*his-6631* (*ubiX*, *dhuA*, *hisJ*, *hisP*)
TA3190 (2)	*dhuA1 hisJ8918*::Tn10	TA3163	*his-6632* (*ubiX*, *dhuA*, *hisJ*, *hisP*)
TA3191 (2)	*dhuA1 hisJ8919*::Tn10	TA3164	*his-6633* (*hisJ*, *hisP*[c])
TA3192 (3)	*dhuA1 hisP6640*::Tn10	TA3179	*dhuA1 his-6638* (*hisJ*, *hisP*)
TA3193 (1)	*dhuA1 hisP6641*::Tn10	TA3181	*dhuA1 his-6639* (*hisJ*, *hisP*)
TA3194 (2)	*dhuA1 hisP6642*::Tn10	TA3077	*his-6794* (*ubiX*, *dhuA*, *hisJ*)
TA3195 (2)	*dhuA1 hisP6643*::Tn10	TA3078	*his-8907 zei-102*::Tn10[d] (*ubiX*, *dhuA*, *hisJ*)
TA3090 (2)	*hisP6757*::Tn10	TA3180	*dhuA1 his-8909* (*hisJ*[e])
		TA3196	*his-8920* (*ubiX*, *dhuA*, *hisJ*)
		TA3197	*his-8921* (*ubiX*, *dhuA*, *hisJ*)
		TA3198	*his-8922* (*ubiX*, *dhuA*, *hisJ*)
		TA3199	*his-8923* (*ubiX*, *dhuA*, *hisJ*)
		TA3200	*his-8924* (*ubiX*, *dhuA*, *hisJ*)

[a] Numbers in parentheses refer to rate of reversion to *D*-histidine growth, determined as described in Methods: 1, 2 and 3 indicate revertant frequencies of about 10^{-7}, 10^{-6} and 10^{-5} respectively

[b] All strains, except NK40 and NK337, carry the deletion mutation *hisF645*

[c] Contains a wild-type *dhuA* site

[d] Presumably contains a partial Tn10 element because TA3078 is Tc^r

[e] This deletion might enter *dhuA* without extending as far as mutation *dhuA1*

lysogenic state in strain NK337 by temperature shift from 30° to 42° (Kleckner et al., 1975). NK337 *(leuA414 sup-19* [P22 Tc-10]), was constructed by N. Kleckner. The titer of the Tc-10 phage preparation was measured by high-frequency transduction of tetracycline-resistance, using the stable lysogen NK40 (*proC* [P22 *sieA6 int3*], obtained from N. Kleckner) as recipient. The phage particle titer, used to calculate multiplicities of infection, was estimated to be 1,000 times this high-frequency transduction titer (personal communication, N. Kleckner).

Selection of Tn10 Insertions. Strains with Tn10 integrated into the *Salmonella* chromosome were selected in two ways. The first method (Kleckner et al., 1975) involved an initial selection for mutants having Tn10 inserted anywhere on the chromosome. A culture of TA271 or wildtype LT2 containing 5×10^9 cells/ml was infected with 4×10^9 P22 Tc-10 phage/ml, and 0.1 ml of the culture was spread on a green indicator plate (Chan et al., 1972) containing 25 μg tetracycline per ml. Tc^r clones arising at 41° were pooled together and infected with P22 phage to obtain a transducing lysate. A *purF* auxotroph (TA1852) was transduced to prototrophy with this lysate. Pur$^+$ Tc^r transductants were then tested for linkage of the Tn10 to *purF* by P22 transduction and for resistance to α-hydrazino imidazole propionic acid (HIPA), a growth-inhibitory histidine analog. Mutants having the mutations *zei-102*::Tn10, *hisP6757*::Tn10, and *hisP6640*::Tn10 in Figure 1 were isolated in this way.

The other Tn10 insertions in *hisJ* and *hisP* were isolated by a direct selection for simultaneous resistance to tetracycline and to HIPA. Resistance to HIPA is the phenotype of *hisP* mutants; *hisJ*::Tn10 mutants are also resistant to HIPA because of polarity on *hisP* expression (Ames et al., 1977). To a suspension of 2.5×10^{10} exponentially growing cells of TA271 (*dhuA1 hisF645*) in 5 ml of L broth, 2×10^{10} P22 Tc-10 phage were added and the culture was shaken for 30 minutes at 37° to allow infection. The culture was then diluted to 100 ml with L broth containing 10 μg/ml tetracycline hydrochloride and 0.01 M EGTA. When the culture reached full growth, a 10 ml aliquot was centrifuged. After being washed once in minimal medium E, the pelleted cells were suspended in 20 ml minimal medium E containing 0.4% glucose, 10^{-4} M HIPA, 10 μg/ml tetracycline hydrochloride, 0.01 mM carnosine (as a source of histidine), and 0.01 M EGTA. After 15 to 20 hours shaking at 37°, 0.1 ml of this culture was spread on minimal agar containing 0.4% glucose, 25 μg/ml tetracycline hydrochloride, 0.01 M EGTA, 0.1 ml of 0.1 M HIPA, and 0.1 ml of 0.01 M carnosine. Colonies arising after two days at 41° were tested for HIPA resistance and tetracycline resistance. Only one such colony was saved from each original culture independently infected with Tc10 phage. In one experiment, under these

conditions, nine out of ten infected cultures yielded a mutant with a Tn10 insertion in *hisJ* or *hisP*. In two previous experiments in which one-fifth as many bacteria (5×10^9) per culture were infected, only one out of four and one out of six cultures yielded such a mutant.

Isolation of Deletion Mutations in the Tn10-Containing Strains. Two approaches were taken, either using a strain in which Tn10 was inserted near the histidine transport operon (strain TA3088: *dhuA1 hisF645 zei-102*::Tn10, Figure 1), or using strains with Tn10 inserted within the histidine transport operon. Starting with TA3088, mutants lacking histidine transport were selected by resistance to O-diazoacetylserine (azaserine), a growth-inhibitory substrate of the transport system (Ames et al., 1977). Azaserine-resistant clones were screened for the loss of Tn10 by testing for sensitivity to tetracycline. Mutations were identified as deletions in the azaserine-resistant, tetracycline-sensitive clones by transductional crosses with strains carrying *hisJ* point mutations. Tc^r strain TA3078 (*hisF645 his-8907*) was identified as a deletion mutant among azaserine-resistant, tetracycline-resistant clones by similar crosses. By this method we selected only for those Tn10-promoted deletions which extended at least to the histidine transport operon.

When the parent strain carried Tn10 inserted within *hisJ* or *hisP*, deletion mutants were isolated by penicillin enrichment for Tc^s cells (Kleckner et al., 1977; Kleckner et al., 1978b). Strains carrying the Tn10 insertion were grown up overnight in nutrient broth and then diluted 100-fold into nutrient broth containing a low concentration of tetracycline hydrochloride (5 μg/ml). When tetracycline-resistance became expressed sufficiently for the bacteria to double about three times, 1.0 ml of this culture was added to 1.0 ml of nutrient broth containing 80,000 units/ml penicillin and 5 μg/ml tetracycline hydrochloride. After incubation for two to three hours, the culture was harvested, diluted into nutrient broth, and incubated to full growth. It was then subjected to a second cycle of penicillin enrichment. Tc^s bacteria in the surviving culture were detected by plating single colonies on green indicator plates (Chan et al., 1972) spread with 0.15 ml of 100 μg/ml tetracycline. On such plates Tc^r bacteria formed white colonies which had a brown center, whereas Tc^s colonies were smaller and had a dark green center (Kleckner et al., 1978b). The presence of deletions in the Tc^s clones was determined by crosses with strains carrying appropriate *hisJ* or *hisP* mutations.

The endpoints of all Tn10-promoted deletions were established by crossing each of them as recipient with appropriate point mutants as donors. All available point mutants (Ames et al., 1977) in the regions defining the endpoints were utilized in these crosses.

Reversion Rate. All insertions in *hisJ* and *hisP* were tested for their ability to revert. Approximately 10^8 bacteria from a saturated nutrient broth culture were plated on a minimal medium petri plate containing 10 μmole of *D*-histidine by the soft agar layer technique (Roth, 1970). After incubation for two days, the number of colonies appearing on the plate were counted. The frequency of reversion is expressed as the number of colonies appearing on these plates per bacterium plated. These colonies represent true revertants deriving from precise excision of Tn10. Presumably, no portion of the Tn10 element is retained in any of them because the integrity of both *hisJ* and *hisP* gene products is essential for growth on *D*-histidine.

Materials. Tetracycline hydrochloride was from Sigma, and Penicillin G was from Parke-Davis. HIPA was synthesized in this laboratory by a procedure previously described (Sletzinger et al., 1968). Azaserine was obtained from the Cancer Chemotherapy National Service Center, National Cancer Institute.

Results

Histidine Transport Genes. The histidine transport genes *dhuA*, *hisJ*, and *hisP* appear to be part of an operon, with *dhuA* as the promoter (Ames et al., 1977). The endpoints of deletion mutations have defined ten regions in *hisJ* and thirteen regions in *hisP* (Fig. 1, and Ames et al., 1977). All of the deletions used for the construction of the map were spontaneous except for *his-6794* (dividing region II from region III in *hisJ*) and *his-8909* (dividing region VIIIb from region VIIIc in *hisJ*), which were Tn10-promoted. No more than one spontaneous deletion ended at each region boundary (Ames et al., 1977): thus, there is no evidence of preferred sites for the endpoints of spontaneously arising deletions.

Tn10 Insertion Mutations. A total of 15 Tn10 insertion mutants have been isolated, each of which resulted from an independent insertion event in the histidine transport operon; only one mutant was saved from each bacterial culture infected by Tc-10 phage. Ten of these insertions map in the *hisJ* gene (Fig. 1). Nine of the *hisJ* insertions map very close together: they are depicted in Figure 1 as being at the boundary of regions II and III of *hisJ*, designated as site A. This boundary is determined by the endpoint of deletion *his-6794*, which is the first Tn10-promoted deletion obtained from insertion *zei-102*::Tn10, in strain TA3088 (Table 1). All of the insertion mutations in site A were crossed against each other, both as donors and as recipients, in transductional crosses selecting for $HisJ^+$ (i.e., *D*-histidine-utilization). No increase in $HisJ^+$ over the expected reversion rate was observed, while all site A insertions recombined freely with the insertion at site B and with the *hisJ* mutants available in regions II and III. Thus, all of these insertions are located at sites extremely close to each other (see Discussion).

The insertion mutations can be divided into three groups according to the frequencies with which they revert to yield the $HisJ^+$ phenotype (Table 1). All of the insertions at site A in the *hisJ* gene, except for *hisJ8912*::Tn10, yield about 200 $HisJ^+$ revertants per 10^8 cells plated, a rather high frequency for precise excision of Tn10 insertion mutations (Kleckner et al., 1978a; Foster, 1977). Insertions *hisJ8912*::Tn10 and *hisP6640*::Tn10 yield about 1,000 revertants per 10^8 cells plated, while *hisP6641*::Tn10 yields only ten $HisJ^+$ revertants per 10^8 cells plated.

The reversion events provided the means of verifying that the tetracycline-resistant $HisJ^-$ mutants were due to Tn10 insertions in the *hisJ* gene, rather than being double mutants having a *hisJ* mutation

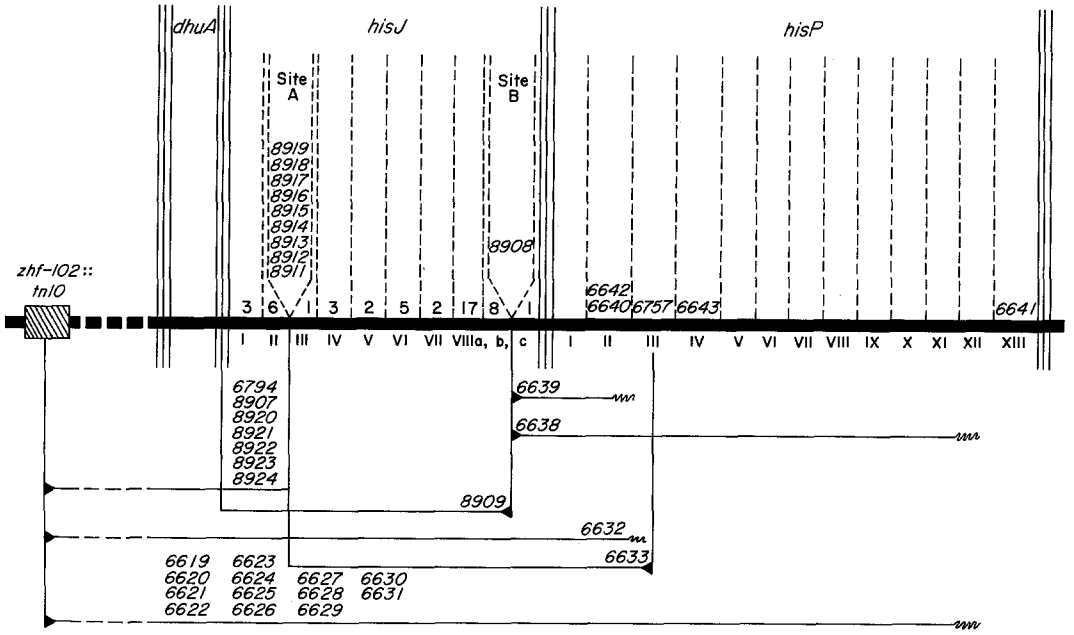

Fig. 1. Tn10-induced mutations in the histidine transport genes *dhuA*, *hisJ*, and *hisP*. Allele numbers listed above the solid line (which represents the chromosome) indicate the positions of separate Tn10 insertion mutations. The lines and allele numbers below the heavy solid line represent Tn10-stimulated deletions. The wiggly portion of the deletion-representing lines indicates uncertain location of that endpoint. The arrowheads are positioned at the presumed origins of the deletions, the sites at which the Tn10 had been inserted. Tn10 insertion mutation *zei-102*::Tn10 is described in the text. The dashed vertical lines indicate the endpoints of spontaneous deletions previously identified in strains not carrying a Tn10 transposon, except for the divisions between *hisJ* regions: II and III, VIIIb and VIIIc, which were defined by Tn10-stimulated deletions. The number of available pointmutant sites in each of the *hisJ* regions I to VIIIc is indicated immediately above the *hisJ* portion of the chromosome (Ames et al., 1977; Noel, 1977)

plus a Tn10 integrated elsewhere on the chromosome. Revertants to $HisJ^+$ were checked for tetracycline-sensitivity, and in every case the $HisJ^+$ revertants were Tc^s. Fifty-nine revertants of insertions at site A were tested: at least two from each insertion and occasionally more. Had the Tn10 actually been inserted elsewhere on the chromosome, all of the $HisJ^+$ revertants would still have been Tc^r.

As shown in Figure 1, five of the Tn10 insertions mapped in *hisP*. Two of these, *hisP6640*::Tn10 and *hisP6642*::Tn10, map within the same region of the gene.

Tn10-Stimulated Deletions. An inserted Tn10 element stimulates the deletion of chromosomal DNA neighboring the site of insertion (Kleckner et al., 1975). One endpoint of such a deletion is in the area of the original Tn10 insertion, perhaps always within the inserted element itself (Kleckner et al., 1978b). The following description will be concerned with the other end of such deletions which is located in the neighboring chromosomal DNA.

Tn10 insertions outside the *hisJ* gene were exploited to obtain deletions which ended within the *hisJ* gene. Most of these deletions were selected in strain TA3088 containing the Tn10 insertion *zei-102*::Tn10, which maps to the left of the *hisJ* gene (Fig. 1) and is 80% linked to *hisJ* by P22 transduction (Ames et al., 1977). Of 21 deletions occurring independently in TA3088 and extending into the histidine transport genes from this insertion, seven end in *hisJ*, one ends in *hisP*, and the other thirteen extend beyond the *hisP* gene. Four more deletion mutations were obtained in strains carrying insertion *hisJ8908*::Tn10 or *hisP6757*::Tn10. Two of these ended in *hisP*, one in *hisJ* and one in the undefined region between *dhuA* and *hisJ*.

Crosses were performed to establish the length of these deletions: the results are shown in Table 2. Of the nine deletions terminating in *hisJ*, eight end at the same site. Moreover, this site coincides with the hot-spot of Tn10 insertion, siteA. Those seven which arose from a Tn10 insertion to the left of the operon (in TA3088) recombine with the single mutation in region III of *hisJ*, but none recombine with any of the six point mutations of region II (Figure 1; Ames et al., 1977). The eighth deletion ending at site A, *his-6633*, having arisen in a strain carrying the Tn10 element in the *hisP* gene (*hisP6757*::Tn10), originates from the opposite

Table 2. Mapping of the Tn10-stimulated deletions

Donor	Tn10-stimulated deletions									Controls	
Recipient (*his*:)	*6794*	*8907*	*8920*	*8921*	*8922*	*8923*	*8924*	*6633*	*6632*	*6766*	*5503*
6 point mutants of *hisJ* region II	0	0	0	0	0	0	0	0–30		>500	
single point mutant of *hisJ* region III	20	20	20	20	15	20	20	0		>500	
control (*hisJ6766*, region VIIIc)	300	400	300	300	200	400	400	0		0	
Tn10 insertions (in hisJ, site A)											
TA3183 (*8911*::Tn10)	1		0	0	0	0	0	0			
TA3184 (*8912*::Tn10)	0		0	0	0	0	0	2			
TA3185 (*8913*::Tn10)	0		0	0	0	0	0	0			
TA3186 (*8914*::Tn10)	0		0	0	0	0	0	0			
TA3187 (*8915*::Tn10)	0		0	0	0	0	0	0			
TA3188 (*8916*::Tn10)	0		0	0	0	0	0	0			
TA3189 (*8917*::Tn10)	0		0	0	0	0	1	0			
TA3190 (*8918*::Tn10)	1		0	0	0	0	0	0			
TA3191 (*8919*::Tn10)	0		0	0	0	0	0	0			
Tn10 insertions (in hisP)											
TA3067 (*6757*::Tn10)								0	100		>500
TA3192 (*6640*::Tn10)								0	0		>500
TA3194 (*6642*::Tn10)								0	0		>500
TA3195 (*6643*::Tn10)								>500	300		>500

Most transductions were executed in spots with 5×10^6 bacterial recipients and 1 to 3×10^8 phage (plaque-forming units). The values presented are the numbers of $HisJ^+HisP^+$ colonies arising in the cross. An empty space means the cross was not done. Control crosses, to indicate the sensitivity of the test, involved *his-6766* (a point mutation in region VIIIc of *hisJ*) and *his-5503* (a deletion of regions XII and XIII of *hisP*). The very small number of colonies encountered in a few of the crosses might indicate a very limited recombination in those cases

direction. It recombines with all the mutations in region II of *hisJ* but not with the single mutation or region III.

None of the eight deletions ending at site A recombined with any of the Tn10 insertion mutations at site A of the *hisJ* gene (Table 2). Thus, the deletions end in the same region of *hisJ* (or extremely close to it) that is a hot-spot for Tn10 insertion.

Other Tn10-stimulated deletions end within the histine transport genes (Fig. 1). Deletions *his-6639* and *his-6632* may end at the same site in *hisP* but have not been intensively mapped. Deletion *his-8909* ends in *dhuA* or *hisJ*, but definitely not at Tn10 site A of *hisJ*. One of the deletion mutants, strain TA3078 (*hisF645 his-8907*), is still Tc^r; it must retain at least part of the Tn10 element. All other deletions are Tc^s.

Imprecise excision of the Tn10 element is accompanied frequently by the occurrence of inversion of neighboring chromosomal regions (Kleckner et al., 1978b). Inversions should behave in a transductional cross like double point mutants if present in the donor. To eliminate the possibility that we were dealing with inversions, all twelve presumed deletions (Figure 1) which had ends within the histidine transport operon and which had already been tested for recombination as recipients (see Methods), were tested also as donors in crosses with appropriate recipient point mutants. All behaved as deletions in these reciprocal crosses, giving recombinants only with mutants whose point mutations are located outside the deleted areas. In agreement with this, all deletions promoted by the Tn10 insertion, *zei-102*::Tn10, are also $UbiX^-$; gene *ubiX* is located between the *zei-102*::Tn10 insertion site and the histidine transport operon (Ames et al., 1977).

According to Kleckner et al. (1978b), in their system inversion events are more frequent than deletion events. This is not in disagreement with our data because our selection and screening were specifically designed to yield deletion mutants. Inversions presubably arose and were discarded.

Discussion

We have shown that Tn10 insertion and the endpoints of Tn10-stimulated deletions are not randomly situa-

ted within the histidine transport genes. A particular region of the *hisJ* gene is selectively involved in these Tn10-associated recombination events. This region has been designated as Tn10 site A (Fig. 1).

Nine of the fifteen Tn10 insertions thus far isolated in the histidine transport genes are at this single site in *hisJ*. Such clustering of Tn10 insertions has been reported previously: a hot-spot for insertion exists in the *hisG* gene in *Salmonella* (Kleckner et al., 1978a), and one has been described in the *lacZ* gene in *E. coli* (Foster, 1977).

Some previous reports have suggested that the endpoints of Tn10-stimulated deletions may be more randomly distributed than insertion sites (Kleckner, 1977). On the other hand, a recent study (Kehoe and Foster, 1977) of a large number of Tn10-promoted deletions in the *tra* operon of plasmid pDU202 present in *E. coli* suggested that the endpoints were non-randomly distributed: the system used in this case did not allow a fine-structure mapping of the endpoints within an individual gene. Our evidence indicates that such deletions ending within *hisJ* have clearly non-random endpoints. Moreover, we show that the preferred endpoints coincide with a hot-spot site for Tn10 insertion. Seven out of eight Tn10-stimulated deletions ending within *hisJ* end at site A. The deletions originate from Tn10 elements inserted on either side of site A. The non-randomness of these deletions is apparently due to the precence of Tn10, because there is no evidence of preferred endpoints among deletions that have been isolated in strains not carrying the Tn10 element (Ames et al., 1977, and Fig. 1).

The physical dimension of site A of *hisJ* cannot be gauged accurately by the genetic mapping utilized in this work. We cannot define the size limits of site A very accurately because we are limited by the density of mutations available in the *hisJ* gene. However, a rough estimate can be made on the basis of the known size of the J protein, which is coded for by the *hisJ* gene, and the number of mutants available in the *hisJ* gene. The J protein has been shown (Lever, 1972) to be a monomer of 25,000 daltons and, from its amino acid analysis, to be composed of about 230 amino acids. Thus, it must be coded for by a gene about 700 mucleotides long. The 78 available *hisJ* mutations map in 48 recombining sites, which are distributed fairly randomly among the ten *hisJ* regions (Ames et al., 1977; Noel, 1977). Thus the mean distance between any two adjacent point mutations is fifteen nucleotides. Site A apparently lies between two adjacent *hisJ* point mutations, and therefore the insertions and the deletion-endpoints within site A might be spread out over about 15 mucleotide residues. Because of the crudeness of these calculations, it is possible that site A is much broader or narrower than our estimate. In the case of the Tn10 insertions in *hisG*, it is suggested that the insertions may not be identical (Kleckner et al., 1978a), which would speak in favor of a larger site of insertion than simply between two particular nucleotides.

What directs insertion and deletion events to this site? One possibility is that Tn10 insertion and deletion formation are catalyzed by the same recombination enzyme, which has relatively great affinity for site A of *hisJ* or an adjacent DNA sequence. A precedent for a factor common to integration and deletion events at specific DNA sites is the *int* gene product of bacteriophages λ or P22. Int$^-$phage are defective in both integration of prophage DNA and the excision from the prophage state (Gottesman and Weisberg, 1971; Levine, 1972). Moreover, there is a class of deletions within λ that occur only in Int$^+$ genetic background, although there is no evidence to indicate that the deletion endpoints are non-random (Davis and Parkinson, 1971). In the case of another translocatable element, ISl, evidence has been presented by Reif and Saedler (1975) that deletions stimulated by ISl in the *gal* region of *E. coli* K12 also have preferred endpoints. They proposed that the deletions are formed by recombinational events between these preferred deletion-endpoint sites and the site of insertion. The behavior of Tn10 in the *hisJ* genetic region conforms to this model proposed for ISl, and further suggests that such preferred sites for endpoints are those DNA sequences which are also preferred sites for Tn10 insertion. The hypothesis of an enzyme common to both Tn10 insertion and deletion events would be bolstered by the discovery of mutations in the Tn10 element (or in the bacterial chromosome) which simultaneously reduce insertions and Tn10-stimulated deletions.

Whatever the basis for the clustering of Tn10-stimulated events at this particular site of *hisJ*, it is important to know whether it represents a general property of the Tn10 element. To this end, other known hot-spots for Tn10 insertion (Kleckner, Roth, and Botstein, 1977; Foster, 1977) can be studied to ascertain whether they are also hot-spots for the ends of Tn10-stimulated deletions. Finally, one might further speculate that transposable elements, such as Tn5, apparently less specific for site of insertion than Tn10 (Kleckner, 1977), also may allow a more random distribution of the deletion endpoints which they induce.

Acknowledgements. We are grateful to F. Ardeshir for isolating three strains critical to this study, strains TA3088 (*dhuA1 hisF645 zei-102*::Tn10), TA3090 (*hisF645 hisP6757*::Tn10), and TA3078 (*hisF645 hisJ8907*). We thank: N. Kleckner, D. Botstein, J. Roth, and S. Kustu for advice and the generous gifts of various strains; K. Nikaido for technical help. This work was supported by NIH agrant AM12121 to G.F.L.A.

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Communicated by A.J. Clark

Received April 4/June 21, 1978