Endpoint Bias in Large Tn10-Catalyzed Inversions in *Salmonella typhimurium*

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ABSTRACT

A genetic strategy identified *Salmonella typhimurium* strains carrying large (>40 kb) Tn10-catalyzed inversions; the inverted segments were characterized by *Xba*I digestion and pulsed field gel electrophoresis. Two size classes of large inversions were found. More than half of the inversions extended 40–80 kb either clockwise or counterclockwise of the original Tn10 site. The remaining inversions extended up to 1620 kb (33% of the genome), but the distal endpoints of these inversions were not randomly scattered throughout the chromosome. Rather, each Tn10 repeatedly yielded similar (though not identical) inversions. The biased endpoint selection may reflect the limited search for target DNA sequences by the Tn10 transposase, and the spatial proximity of the donor and target regions in the folded *S. typhimurium* nucleoid. Using this interpretation, the data suggest that DNA sequences 40–80 kb clockwise and counterclockwise of the insertion site are in spatial proximity with the insertion, perhaps reflecting the organization of DNA into ~120-kb nucleoid domains. In addition, the data predict the spatial proximity of several distant DNA regions, including DNA sequences equidistant from the origin of DNA replication.

THE transposable element Tn10 can catalyze the inversion or deletion of DNA sequences adjacent to the insertion site (ROSS *et al.* 1979; KLECKNER *et al.* 1979; KLECKNER 1989). These intramolecular transposition events require the interaction between the IS10-encoded transposase, the inside ends of the two IS10 elements, and a distant DNA target. Cleavage of the inside ends of the IS10 element and ligation with the target DNA result in loss of the tetracycline-resistance genes, and formation of an adjacent chromosomal deletion or inversion (see Figure 1). Previous work has shown that 90% of the inversions catalyzed by Tn10 are caused by exchanges at target sites less than ~45 kb from the site of the Tn10 insertion; the larger inversions have not been previously characterized (KLECKNER *et al.* 1979; KLECKNER 1989; SHEN *et al.* 1987). While the size of a deletion is limited by the locations of essential genes, inversions are not subject to this size limitation.

The Tn10-catalyzed inversions and deletions have relatively simple enzymatic requirements that have been defined both *in vivo* and *in vitro*. For inversion or deletion formation, the IS10-encoded transposase performs all of the required cleavage and ligation reactions, using the inside IS10 ends. Host factors, such as IHF and HU proteins, are not required *in vitro* when the inside ends of IS10 are used, nor do mutations in the genes encoding these proteins affect the frequency of IS10-catalyzed inversions *in vivo* (MORISATO and KLECKNER 1987;

ROBERTS *et al.* 1985). The activity of the IS10 ends, and transcription from the transposase promoter are both stimulated by hemi-methylated DNA, which will exist just after the replication fork passes the Tn10 site, and before the Dam-system can re-methylate the DNA (CAMPBELL and KLECKNER 1988, 1990; MORISATO and KLECKNER 1987).

The transposase enzyme is not freely diffusible *in vivo*. Synthesized from IS10-right, the transposase preferentially binds to nearby IS10 ends, and has difficulty complementing in “*trans*.” As the IS10 ends are separated, transposition activity decreases about 40% per kilobase (MORISATO *et al.* 1983). Other bacterial transposons, such as Tn5 and IS903, have similarly “*cis*-acting” transposases (DELONG and SYVANEN 1991; DERBYSHIRE *et al.* 1990). The chromosomal target site selected in Tn10-mediated inversions is also preferentially nearby. Most of the inversions generated from a *hisG*:Tn10 insertion were within ~45 kb of the insertion site (KLECKNER *et al.* 1979). Selection of a target site for IS10 transposition lacks this preference for nearby DNA, perhaps resulting from excision of the IS10-transposase complex and diffusion of the complex to the chromosomal target (SHEN *et al.* 1987).

The organization of the bacterial nucleoid will result in the juxtaposition of DNA sequences that are not linearly contiguous. No direct experiments have yet shown whether spatially neighboring DNA sequences provide “nearby” targets for the transposase. If the search for target sites is within a limited volume surrounding the insertion, then large Tn10-catalyzed rearrangements would be expected to reflect this limited search. If the folded bacterial nucleoid is sufficiently organized, then a limited search for distant target sites would be expected to produce a bias in the

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Deletion

Inversion

FIGURE 1.—*Tn10*-mediated rearrangements. *Tn10* catalyzes deletion or inversion of adjacent chromosomal sequences, using the inside ends of the *IS10* elements. In both of these events, the sequences between the *IS10* elements are deleted. Thus, the *Tn10*-catalyzed inversions also delete the tetracycline-resistance genes, and are also known as “inversion/deletions” (KLECKNER *et al.* 1979). For simplicity, these events are referred to as “inversions” in the text.

endpoints of large transposase-catalyzed rearrangements. This bias could provide clues to the folding pattern of the *in vivo* nucleoid.

Large inversions formed by *Tn10* may provide a genetic tool to assess *in vivo* nucleoid structure. The endpoints of large *Tn10*-catalyzed inversions will document the DNA target sequences most frequently able to physically interact with the *IS10* inside ends during the time when the transposase is active. The temporal regulation of the *Tn10* transposase may provide a technical advantage if the DNA folding pattern changes through the cell cycle. No evidence yet shows whether the organization of DNA in the bacterial nucleoid is random, fixed, or dynamic. If the DNA is randomly arranged in different cells, or if the DNA is sufficiently flexible, all *Tn10*-catalyzed inversion endpoints will be equally likely, whether or not there is a limited volume search for target sites by the transposase. However, we have found that the endpoints of large *Tn10*-catalyzed inversions are highly biased.

MATERIALS AND METHODS

Strains: All strains were derived from *Salmonella typhimurium* strain LT2, and were stored at -80° in 0.8% dimethyl sulfoxide (DMSO). The parental strains for these experiments were made by transducing *Tn10* insertions into the strain SE5017, which carries the *leu-485* mutation. Table 1 lists the parental strains.

Media: Fusaric acid medium for selection of tetracycline-sensitive strains was made according to MALOY and NUNN (1981). Growth of strains in liquid culture was in LB medium (5 g yeast extract, 10 g Bacto-tryptone, 5 g NaCl) at 37° . In solid medium, LB was supplemented with 15 μ g/ml tetracycline when needed. The E medium of VOGEL and BONNER (1956) was used for solid minimal medium and supplemented with individual nutrients as necessary. Solid media were made with 1.5% agar (Difco).

TABLE 1

Summary of *Xba*I digests

Parental strain	Insertion	Total	<i>Xba</i> I digest pattern			
			WT	Novel	Del	Odd
SE7970	<i>trp-1042::Tn10</i>	21	16	5	0	0
SE7971	<i>aroD553::Tn10</i>	8	0	7	1	0
SE8980	<i>pyrA2276::Tn10</i>	10	8	2	0	0
SE8981	<i>pyrA2277::Tn10</i>	10	4	3	2	1
SE7968	<i>purD877::Tn10</i>	7	1	5	1	0

Columns 1 and 2: parental strain number and *Tn10* insertion. Each of these strains also carries the *leu-485* mutation. Column 3: the total number of independently derived large rearrangements. Columns 4–8: number of strains with wild-type *Xba*I pattern (WT); novel *Xba*I pattern but wild type amount of DNA (Novel); *Xba*I pattern interpreted as an adjacent deletion (Del); or uninterpretable *Xba*I patterns (Odd). Strains with wild-type or novel *Xba*I patterns were shown to carry large inversions.

Selection of tetracycline-sensitive strains: Cells from frozen (-80°) stocks of the parental *Tn10*-containing strains were streaked onto LB agar and incubated overnight. From these plates, ten colonies per strain were picked and cultured overnight in LB broth to create independent cultures. These cultures were diluted 100-fold, and aliquots were plated onto fusaric acid medium plates (MALOY and NUNN 1981), and incubated for 48 hr at 37° . Putative tetracycline-sensitive colonies were purified once by streaking onto fusaric acid medium, then tested for tetracycline resistance or sensitivity. Overnight cultures of the tetracycline-sensitive derivatives were used in “spot transductions,” which tested the frequency of transductional repair of the *Tn10*-induced auxotrophy. For these spot transductions, E plates supplemented with leucine were seeded with 0.1 ml of P22 HTint grown on a wild-type host ($\sim 10^9$ pfu/ml), then spotted with 0.02 ml of overnight cultures, and incubated 36 hr at 37° . The distinction between wild-type transduction frequencies and low transduction frequencies was generally very clear from the spot tests. Several infrequently transduced strains from each independent culture were retested by full-plate transduction crosses. From each independent culture, one strain with low transduction frequency (less than 10% of the wild-type number of transductants) was chosen and saved by storage in 0.8% DMSO at -80° . The remaining strains were discarded. In all cases, transduction of other markers (such as the *leu-485* mutation) occurred at wild-type frequencies.

Isolation of high molecular weight DNA: The protocol accompanying the New England BioLabs ImBed Kit (catalog no. 375) was slightly modified. Overnight cultures (3 ml) were grown at 37° in LB broth, then spun down and resuspended in 0.5–1 ml “cell suspension solution” (10 mM Tris-HCl pH 7.2, 20 mM NaCl, 100 mM EDTA). Equal volumes (0.5 ml each) of resuspended cells and 1.6% molten low melting point agarose (NuSieveGTC, FMC BioProducts) were mixed together, drawn into a 1-ml syringe, then allowed to harden 15–30 min at room temperature. The agarose-cells plug was transferred to one well of a 6-well microtiter dish (Corning no. 25810-6), and cut into three pieces for easier handling. Lysozyme solution (4 ml of 1 mg/ml lysozyme in 10 mM Tris-HCl pH 7.2, 50 mM NaCl, 100 mM EDTA, 0.2% Na^+ -deoxycholate, 0.5% *N*-laurylsarcosine- Na^+) was added to each well, incubated 2 hr at 37° , then aspirated from the wells. “Wash solution” (4 ml of 20 mM Tris-HCl, pH 8.0, 50 mM EDTA) was added, incubated 15 min at room temperature, and then removed by aspiration; this step was repeated once. Proteinase K solution (4 ml of 1 mg/ml proteinase K in 100 mM EDTA, 1% *N*-laurylsarcosine-

Na⁺, 0.2% Na-deoxycholate) was added, incubated 18 hr at 50°, then removed by aspiration. The plugs were washed with 4 ml wash solution (30 min at room temperature), then with wash solution with added phenylmethylsulfonyl fluoride (PMSF) (4 ml wash solution + 40 µl 0.1 M PMSF; 1 hr at room temperature in a fume hood). Following this, three additional 4-ml incubations in wash solution (30 min each at room temperature), and two incubations (4 ml, 30 min at room temperature) in "storage solution" (2 mM Tris-HCl, pH 8.0, 5 mM EDTA), were performed. The DNA-agarose plugs were then stored in 5 ml storage solution in the microtiter wells at 4°. The microtiter box was sealed with parafilm, and DNA in the plugs was found to be stable for (at least) several months.

Restriction digestion: A ~0.5-mm (~10 µl) slice of the DNA-agarose plug was cut with a sterile scalpel blade, then transferred into 15 µl restriction enzyme buffer mix (total volume, ~25 µl). The DNA-agarose slice was preequilibrated on ice 15–30 min. The gel slice was removed with a spatula and transferred to a 50-µl reaction mix (total volume, 60 µl). The agarose was melted in a heat block for 10 min at 70°. (This step mixed the buffer and DNA and also inactivated endogenous nucleases still remaining in the plug. While we took precautions to minimize shearing in all steps, in our hands, this melting step was necessary to get good digestion without degradation of the DNA. Such melting resulted in no obvious DNA degradation in our hands.) Restriction enzyme was added [*Xba*I (New England BioLabs), 3 µl (20 units/µl); *Bln*I (Takara Biochemicals), 1 µl (8 units/µl)]. Digestion was at 37° for 4 hr. The plugs were again melted at 70° for 5 min just before loading. Pipetting was done carefully with "pipetman"-style pipetors, with the plastic tips cut to decrease shearing during pipetting. The wells were loaded (25 µl/well), then sealed with a drop of 1.6% low melting point agarose. Restricted DNA was found to be stable for at least 2 weeks when stored at 4°.

In the course of these experiments, we found that LT2-derived strains, and *leu-485*-derived strains had single *Xba*I and *Bln*I band differences. The *Bln*I "B" band of LT2 (900 kb, WONG and MCCLELLAND 1992) was 850 kb in strains derived from a *leu-485* background, while the *Xba*I "H" band is 230 kb in LT2 strains and 240 kb in *leu-485* strains. This is consistent with at least one genetic difference (the *leu-485* background has a *glpR*-constitutive phenotype) that we have noted between these two strain backgrounds (SPRINGER 1993). Potentially, the *GlpR* phenotypic change and the *Xba*I band change arise from the same mutation. In addition, we found that strains carrying a *dam* mutation have several additional *Xba*I cleavages. The *Xba*I site (TCTAGA) could overlap some of the *dam* methylation sequences (GATC), preventing cleavage at all of the *Xba*I sites in wild-type strains.

Pulsed field gel electrophoresis: Agarose gels (1%, Sigma no. A-6013) were made with 0.5-cm wells, with 0.5 × TBE buffer (SAMBROOK *et al.* 1989). The gels were run at 14C according to recommended pulse times/run times from a ChefMapper apparatus (Bio-Rad). Both ChefMapper apparatus and Bio-Rad CheffII apparatus were used. Gels were stained with ethidium bromide (30 min), then destained and photographed.

Probability of distal inversion endpoint selection: Each inversion was treated as an independent Bernoulli trial, with all chromosomal sites equally likely to serve as an inversion endpoint. With five very large *trp* inversions, the probability of three or more endpoints landing within one 80-kb segment was calculated as a conditional probability: *i.e.*, given the first endpoint ($P = 1.0$) what is the probability that two (or more) of the four remaining inversion endpoints will end within the same 80-kb interval?

This is given as:

$$\sum_{k=2}^4 \binom{4}{k} (p)^k (1-p)^{4-k}$$

(LARSEN and MARX 1986). For these calculations, we have conservatively assumed that 50% of the chromosome (~2400 kb) is "permissive" for inversion formation (SEGALL *et al.* 1988), thus, $P = 80 \text{ kb}/2400 \text{ kb}$, and the likelihood that the *trp* inversion endpoints occurred at random is 6×10^{-3} . If a larger percentage of the chromosome is permissive for inversion formation, the inversion endpoint bias becomes more extreme, and the observed inversion endpoint distribution is less likely to have occurred at random.

RESULTS

Isolation of strains carrying Tn10-induced large chromosomal rearrangements: Isogenic parental strains were constructed, each with a different well characterized Tn10 insertion, conferring both tetracycline resistance and a nutritional auxotrophy (see Table 1). Tetracycline-sensitive derivatives were isolated from each parental strain by selection on fusaric acid medium, upon which tetracycline-resistant strains cannot grow (BOCHNER *et al.* 1980; MALOY and NUNN 1981). The tetracycline-sensitive derivatives occurred spontaneously at a frequency of approximately 10^{-4} , consistent with previous measurements (KLECKNER *et al.* 1979).

Tetracycline-sensitive strains harboring large chromosomal rearrangements (either deletions or inversions) were distinguished from other tetracycline-sensitive strains (point mutations, excisions, small inversions or small deletions) by a generalized transduction assay (see Figure 2). In these experiments "large" deletions or inversions were defined by the size of the P22 transduced fragment, approximately 45 kb. Strains carrying deletions or inversions larger than the size of a single transduced fragment will yield few *Trp*⁺ transductants, since they require two transduced fragments for repair, a relatively infrequent event with sufficiently dilute transducing lysate (SCHMID and ROTH 1983a, b). Roughly 10% of the tetracycline-sensitive strains became much more difficult to transduce to prototrophy, suggesting that they had acquired large Tn10-induced chromosome rearrangements when they lost tetracycline resistance. Independently derived strains showing reduced transduction to prototrophy were saved for further analysis.

Analysis of inversion restriction patterns: The nature and size of the chromosomal rearrangements were documented by *Xba*I or *Bln*I digestion and pulsed field gel electrophoresis. Both the *Xba*I and *Bln*I restriction maps of *S. typhimurium* have been determined (LIU and SANDERSON 1992; WONG and MCCLELLAND 1992).

Because the Tn10 element has a single *Xba*I site, a comparison of the *Xba*I pattern from a wild-type strain and the pattern from the parental Tn10-containing strain determined the precise physical location of each Tn10 insertion. For example, the *trp-1042::Tn10* inser-

A Isolation & characterization of large rearrangements

B Test of transduction to *Trp*⁺

C XbaI fragment patterns

FIGURE 2.—(A) Method for identification of large rearrangements generated by *trp::Tn10*. (B) Either deletions or inversions larger than the size of a P22 transduced fragment (~45 kb) will be infrequently repaired by generalized transduction. (C) *Trp* lies within the *XbaI* “D” band. The *XbaI* site within *Tn10* is lost, and *BlnI* sites within each *IS10* element remain, after deletion or inversion. In the first inversion example, the inverted segment (shaded region) lies within the “D” band and yields a wild-type *XbaI* pattern, but a novel *BlnI* pattern, since *IS10* elements bracket the inverted segment. In the second inversion example, an inversion between the *trp* site and a site in the *XbaI* “A” band yields a novel *XbaI* fragment pattern.

FIGURE 3.—*XbaI* digestion and pulsed field gel electrophoresis of strains derived from *trp-1042::Tn10*. Lanes 1 and 2, λ ladder (Bio-Rad) and *Saccharomyces cerevisiae* chromosomes (Bio-Rad); lane 3, SE7970 (*trp-1042::Tn10*); lane 4, SE5017 (*leu-485*); lanes 5–8, inversion strains SE8870, SE8868, SE8874, SE8872. Electrophoretic conditions: Bio-Rad Chef-Mapper, ramped pulse time: 24–79 sec; run time: 38 hr; 14°.

tion is located 120kb from one end of the *XbaI* “D” fragment; strains carrying this insertion have novel 120- and 340-kb *XbaI* fragments, and have lost the wild-type 457-kb *XbaI* “D” fragment (compare Figure 3, lanes 3 and 4).

Rearrangements catalyzed by *Tn10* will change the *XbaI* fragment pattern. Removal of the tetracycline-resistance genes will cause loss of the *XbaI* site of *Tn10*, restoring a wild-type *XbaI* pattern. However, the loss of DNA through deletion will be apparent in the *XbaI* pattern, as a smaller size of the band harboring the insertion, or as fusion of two adjacent bands. An inversion will preserve the total amount of DNA (except for the deletion of the tetracycline-resistance genes), but may alter the distance between some restriction sites. Figure 2C diagrams possible *XbaI* fragment changes that were expected to result from large *Tn10*-catalyzed inversions and deletions. All of the independently derived tetracycline-sensitive strains showing reduced transductional repair were screened by *XbaI* digestion and pulsed field gel electrophoresis. Of 56 independent *Tn10*-mediated large rearrangements, approximately 90% were interpreted as simple inversions based on their *XbaI* pattern (see Table 1).

DNA is not lost or gained in strains carrying large *Tn10*-induced inversions: Approximately 60% of the strains satisfying the genetic criteria for large inversions or deletions had wild-type *XbaI* patterns, a result that was

TABLE 2
Characteristics of individual inversions

Strain	Parental Tn10	Missing WT XbaI bands	Novel XbaI bands (kb)	Inversion size (kb)
SE8859	<i>trp-1042::Tn10</i>	None	None	85 (CCW) ^a
SE8868	<i>trp-1042::Tn10</i>	A, D	440, 825	510
SE8869	<i>trp-1042::Tn10</i>	A, D	480, 775	555
SE8870	<i>trp-1042::Tn10</i>	None	None	50 (CCW) ^a
SE8871	<i>trp-1042::Tn10</i>	None	None	60 (CCW) ^a
SE8872	<i>trp-1042::Tn10</i>	A, D	380, 880	953
SE8873	<i>trp-1042::Tn10</i>	None	None	35 (CCW) ^a
SE8874	<i>trp-1042::Tn10</i>	A, D	400, 860	473
SE9100	<i>trp-1042::Tn10</i>	C, D	570, 130	353
SE8860	<i>aroD553::Tn10</i>	A, Q	70, 770	76
SE8861	<i>aroD553::Tn10</i>	C, Q	60, 650	1448
SE8862	<i>aroD553::Tn10</i>	G, Q	55, [240] ^b	59
SE8863	<i>aroD553::Tn10</i>	A, Q	44, 810	43
SE8864	<i>aroD553::Tn10</i>	C, Q	140, 570	1528
SE8865	<i>aroD553::Tn10</i>	G, Q	43, 250	43
SE8866	<i>aroD553::Tn10</i>	G, Q	57, 235	59
SE9010	<i>pyrA2276::Tn10</i>	A, B	640, 850	1057
SE9011	<i>pyrA2276::Tn10</i>	None	None	280 (CW) ^a
SE9013	<i>pyrA2276::Tn10</i>	None	None	45 (CW) ^a
SE9014	<i>pyrA2276::Tn10</i>	None	None	45 (CW) ^a
SE9015	<i>pyrA2276::Tn10</i>	None	None	40 (CW or CCW) ^a
SE9053	<i>pyrA2276::Tn10</i>	E, B	58, 470	184
SE9018	<i>pyrA2277::Tn10</i>	A, B	1060, 430	847
SE9019	<i>pyrA2277::Tn10</i>	None	None	60 (CCW) ^a
SE9022	<i>pyrA2277::Tn10</i>	None	None	50 (CCW) ^a
SE9023	<i>pyrA2277::Tn10</i>	C, B	840, 540	1618
SE9024	<i>pyrA2277::Tn10</i>	None	None	120 ^c (CW) ^a
SE9025	<i>pyrA2277::Tn10</i>	A, B	500, 980	922
SE9054	<i>pyrA2277::Tn10</i>	None	None	50 (CCW) ^a
SE8893	<i>purD877::Tn10</i>	E, F	95, 550	313
SE8894	<i>purD877::Tn10</i>	E, F	107, 530	297
SE8895	<i>purD877::Tn10</i>	E, F	112, 530	304
SE8896	<i>purD877::Tn10</i>	E, F	350, 300	60 (or 110) ^c
SE8899	<i>purD877::Tn10</i>	E, F	112, 530	304

Columns 1 and 2: strain number of the inversion, and Tn10 insertion in the parent strain. The alphabetic name of the missing wild type bands (LIU and SANDERSON 1992), and sizes of the novel bands are given for each strain. All other bands appeared identical to wild type. The calculated size of the inversion, based on the XbaI data, is given in the last column.

^a Based on BlnI digestion data, and the wild-type XbaI pattern.

^b Strain SE8862 had only one novel XbaI band. Another band of 240 kb was expected, but not definitively observed within the cluster of four wild-type XbaI bands in the 243–224 kb size range.

^c Ambiguity remains regarding the size of this inversion.

predicted for inversions that lay entirely within the XbaI band of the parental Tn10 (see Figure 2). One strain with a wild-type XbaI pattern, derived from *trp-1042::Tn10*, is shown in Figure 3, lane 5. This inversion can extend from the *trp* site either clockwise (less than 340 kb) or counterclockwise (less than 120 kb), yet remain entirely within the XbaI “D” band. Such inversions will satisfy the genetic “transduction test,” because the inversions are larger than 45 kb. There was no evidence of DNA loss or gain in any of these strains (other than the loss of the tetracycline resistance genes), since in all cases, the XbaI patterns were identical to wild type. BlnI digestion confirmed that many of these strains carried large inversions, and allowed determination of the end-point locations of many of the *trp::Tn10*-induced and *pyrA::Tn10*-induced inversions that had wild-type XbaI fragment patterns (see below).

Two novel XbaI bands were expected in strains carrying sufficiently large inversions. Five strains derived from *trp-*

1042::Tn10 had novel XbaI fragments. In four of these strains, the wild-type XbaI “A” and “D” bands were missing, and two new bands appeared. No other bands differed from wild type. The sum of the new band sizes was 1260 kb, identical to the sum of the wild-type XbaI “A” (800 kb) and “D” (457 kb) band sizes (see Figure 3, lanes 5–8). These patterns were expected of inversions between the *trp::Tn10* site (in XbaI band “D”), and sites within the XbaI “A” band. Within experimental measurement, the sum of the novel band sizes was equal to the sum of the missing wild-type band sizes in all of the *trp::Tn10*-induced inversion strains as well as those derived from *pyrA2276::Tn10*, *pyrA2277::Tn10*, *aroD553::Tn10*, and *purD877::Tn10* (see Table 2). Thus, the Tn10-catalyzed inversions that were characterized in this study showed no evidence of additional chromosome rearrangements, nor gross deletion or addition of DNA.

Distal endpoints of large inversion strains: The novel XbaI fragment patterns allowed more precise localiza-

tion of the distal inversion endpoints. The *Xba*I fragment patterns of three *trp-1042::Tn10*-derived inversions are shown in Figure 3, lanes 6–8. The physical location of the *trp-1042::Tn10*, and the sizes of the two novel bands were sufficient to calculate the location of the distal inversion endpoint with a twofold ambiguity. This twofold ambiguity occurred in the analysis of many of the inversions. In the *trp* inversions, for example, the ambiguity arose because the data did not distinguish whether band D gained 20 kb to become the novel 480-kb band, or whether band D gained 320 kb to become the novel 780-kb band. Either of two methods was used to resolve the ambiguity. Very often, the combined data from the pattern of *Xba*I digestion and the pattern of *Bln*I digestion were sufficient to determine which one of the two possibilities was correct. Alternatively, a *Tn10* insertion was introduced into the inversion strains to provide a new *Xba*I site. The location of the *Tn10* was chosen so that *Xba*I would cleave one of the two novel inversion *Xba*I fragments. In some cases, the sizes of the fragments and location of the parental *Tn10* insertion allowed unambiguous determination of the inversion endpoint, and in a few cases, the ambiguity was not resolved.

In general, only the endpoints of the large inversions with novel *Xba*I patterns were determined, overall, about 40% of the large rearrangement strains. However, the percentage varied with each chromosomal site, depending on the size of the parental *Xba*I band, and the location of the *Tn10* insertion within the *Xba*I band. The inversions generated by *aroD553::Tn10* provided a unique case in which no wild-type *Xba*I patterns were expected. The *aroD553::Tn10* insertion resides in the middle of the 49-kb *Xba*I “Q” band, which made it unlikely that an inversion internal to the “Q” band would satisfy the genetic criterion of non-transducibility. Of the eight *aroD* rearrangements analyzed, all had patterns different from wild type. Seven were interpreted as inversions, because two wild-type bands were missing, and two novel bands were detected. One strain was interpreted as a deletion, since the wild-type “Q” band was missing, but no other band changes were detected. Five of the *aroD::Tn10*-catalyzed inversions were relatively small—two extended counterclockwise 40–80 kb, while three extend clockwise 40–60 kb. Two other *aroD::Tn10*-catalyzed inversions (in strains SE8861 and SE8864) extended approximately 1450–1530 kb clockwise of *aroD*, ending within the *Xba*I “C” band. Remarkably, the distal endpoints of these large inversions were within 80 kb of one another, but were clearly not at the same site, as was obvious from the difference in novel band sizes (see Table 2).

The distal endpoints of the remaining large inversions were determined and suggested that each transposon had a small number of preferred distant target regions. Four of five *purD877::Tn10*-derived inversions extended 297–313 kb from the *purD* location (*Xba*I “F”

band), into the neighboring *Xba*I “E” band. Three of five *trp-1042::Tn10*-derived inversions extended 473–555 kb from the *trp* site (*Xba*I “D” band) into the *Xba*I “A” band. Three of five *pyrA::Tn10*-derived inversions extended 847–1057 kb into the *Xba*I “A” band. The *pyrA::Tn10*-derived large inversions originated from two *pyrA::Tn10* insertions, known to have different sites of insertion. This suggests that the chromosomal location of the *Tn10*, rather than the specific insertion site, determined the preferred endpoint region. These results are listed in Table 2, and summarized graphically in Figure 4. Since the genetic criteria demanded only that the distal inversion endpoint lay more than ~45 kb from the site of insertion, the bias in distal endpoint location was surprising. The possible causes of this bias are explored further in the DISCUSSION.

Strains with wild-type *Xba*I patterns have altered *Bln*I patterns: Almost 60% of the *Tn10*-induced rearrangement strains had *Xba*I digestion patterns that were identical to wild type. To verify that these strains carried large inversions, the *Bln*I fragment patterns of several strains with wild-type *Xba*I patterns were determined. The enzyme *Bln*I cleaves within both *IS10* elements. Thus, the *Bln*I pattern observed in the parental *Tn10*-containing strain should lack one wild-type fragment, and should have two novel fragments generated by *Bln*I cleavage at the site of the *Tn10*. (The ~8-kb *Tn10* fragment between the *IS10* insertions will not be seen in these gels.) In strains carrying a *Tn10*-mediated inversion, *IS10* elements will bracket the inverted segment (see Figure 1), resulting in loss of at least one parental *Bln*I band, and the gain of two new *Bln*I fragments. The combined data from the *Xba*I digestion, and *Bln*I digestion were often sufficient to unambiguously determine the size of the inverted segment.

The *Bln*I patterns of several *trp::Tn10*-derived and *pyrA::Tn10*-derived inversions with wild-type *Xba*I patterns were determined, and the sizes of novel *Bln*I bands are given in Table 3. Most of the *pyrA::Tn10*-derived inversions were relatively short; four extended 40–60 kb counterclockwise of *pyrA*, and four extended 45, 45, 120 and 280 kb clockwise of *pyrA*. Likewise, four of the *trp::Tn10*-derived inversions with wild-type *Xba*I patterns were found to extend 35–85 kb, although all of these extended counterclockwise of the *trp* locus.

The *Bln*I patterns of the larger *trp-1042::Tn10*-derived inversions (Table 3B) and *aroD553::Tn10*-derived inversions (data not shown) were determined. In all cases, these results were consistent with our interpretation of the inversion size based on the *Xba*I fragment patterns.

Additional *IS10* transposition accompanies many large inversions: The *Bln*I digestion patterns of 6 of 16 inversion strains showed an extra *Bln*I cleavage site, in addition to those caused by the *IS10* elements bracketing the inversion (see Table 3). The loss of a *Bln*I band

FIGURE 4.—Maps showing inversion endpoints. The arcs outside the *Xba*I chromosomal restriction maps indicate the inverted segments. Also shown (lower left) is a closeup view of the inversion endpoints within the *Xba*I “A” and “C” bands. A composite view (lower right) shows the relationship of the inversion endpoints to the origin and terminus of DNA replication. The position of *aroD553::Tn10* differs from the position that would be inferred from the most recent *S. typhimurium* genetic map (SANDERSON and ROTH 1988), but is consistent with the map position of *aroD* determined by BENSON and GOLDMAN (1992). The positions of all parental insertions were confirmed by the *Xba*I digestion pattern of the parental strains.

unlinked to either the parental *Tn10* insertion, or the inverted segment, and the appearance of two new bands whose sizes summed to the size of the lost band, suggested that these strains carried an additional *IS10* element. Previous experiments documented that an additional *IS10* transposition accompanied about 15% of *Tn10*-induced chromosome rearrangements (RALEIGH and KLECKNER 1984). We found that almost 40% (6/16) of the *trp-1042* and *pyrA::Tn10*-derived large inversions had an extra *Bln*I cleavage site unlinked to either the initial *Tn10* site or the distal inversion endpoint. The *Bln*I bands disrupted by the putative *IS10* insertions are listed in Table 3.

Probability of the observed bias: The endpoints of very large inversions appeared clustered into preferred regions. We estimated the likelihood that the clustered inversion endpoints arose randomly (see MATERIALS AND METHODS). Three of the five very large inversions catalyzed by the *trp-1042::Tn10* insertion lay in the *Xba*I “A” fragment within 80 kb of one another.

If the endpoints were chosen at random, and if at least half of the chromosomal sites can give a viable inversion (see DISCUSSION), then the probability that three endpoints would occur within an 80-kb interval is less than 6×10^{-3} . Using similar assumptions, the likelihood that the very long inversion endpoints of *aroD*, *purD* and *pyrA* occurred by random selection, ranges from 2×10^{-2} to 1×10^{-6} .

DISCUSSION

In previous studies, large bacterial inversions were generated by recombination between homologous sequences engineered into different chromosomal sites (REBOLLO *et al.* 1988; SEGALL *et al.* 1988). In these experiments, some, but not all, pairs of chromosomal sites yielded inversions, and these results defined “permissive” and “non-permissive” inversion intervals (see also SEGALL and ROTH 1989; MAHAN and ROTH 1991). These intervals occurred as large contiguous blocks. When the

TABLE 3
Results of *BlnI* digestion

Strain	Missing WT <i>BlnI</i> bands	Novel <i>BlnI</i> bands (kb)	Interpretation
A. Derived from <i>pyrA2276::Tn10</i> (SE8980)			
SE8980	A	1075, 550	Parental <i>Tn10</i>
SE9010	A, D	1050, 550, 45, (520)	INV (1050), <i>IS10</i> (D)
SE9011	A	550, 280, (800)	INV (280)
SE9013	A	1030, 550, (45)	INV (45)
SE9014	A	1030, 550, (45)	INV (45)
SE9015	A	1030, 510, (40, 45)	INV (40 or 45), <i>IS10</i> (A)
SE9019	A	1075, 490, (60)	INV (60)
SE9022	A, C	1075, 550, 500, 275, (50)	INV (50), <i>IS10</i> (C)
SE9024	A, F2	950, 550, 120, (X, Y ^a)	INV (120 ^a), <i>IS10</i> (F2)
SE9054	A	1075, 500, (50)	INV (50)
B. Derived from <i>trp-1042::Tn10</i> (SE7970)			
SE7970	C	695, 95	Parental <i>Tn10</i>
SE8859	C	610, 95, (85)	INV (85)
SE8868	C	510, 185, 95	INV (510)
SE8870	C	645, 95, 50	INV (50)
SE8871	C, B	640, 460, 350, 95, 60	INV (60), <i>IS10</i> (B)
SE8872	C, A	695, 200, 1400, 95	INV (895)
SE8873	C	660, 95, 35	INV (35)
SE8874	C, B	480, 460, 360, 220, 95	INV (480), <i>IS10</i> (B)

(A) *pyrA2276::Tn10*-derived inversions that had wild-type *XbaI* digestion patterns. (B) *trp-1042::Tn10*-derived inversions. The alphabetic name of the wild-type *BlnI* band(s) missing in each strain is given in column 2. Column 3 lists the sizes of novel *BlnI* bands. Bands that were expected, but not observed are indicated in parentheses. These bands were either too small to remain on the gels that were run or of similar size to one of the wild-type bands, which prevented the recognition of a novel band. The last column provides the interpretation of the *BlnI* digestion data, giving the inversion size (in parentheses), and the location of any additional *IS10* element (with the affected *BlnI* band in parentheses). Due to the wide range of sizes of the *BlnI* bands, the accuracy of the band sizes is less than the accuracy of the *XbaI* band sizes. Wild-type *BlnI* band sizes (in kb) are: A (1600); B (900); C (790); D (570); E (530); F1 (190); F2 (150). The order of *BlnI* fragments is given in WONG and McCLELLAND (1992).

^a The size of this inversion is uncertain. Two bands derived from F2 cleavage were expected, but not observed.

his operon was tested, 55% of the chromosome was included in a contiguous permissive arc, while 40% of the chromosome was in a contiguous non-permissive arc (SEGALL and ROTH 1989).

All of the *Tn10*-generated inversions described here lay within "permissive" inversion intervals that were established by SEGALL *et al.* (1988). The natural inversion that distinguishes the order of genes in the terminus regions of *Escherichia coli* and *S. typhimurium* made comparison with the data of REBOLLO *et al.* (1988) more difficult. However, the 40–60-kb *aroD::Tn10* inversions, and the very large (~500 kb) *trp::Tn10* inversions are similar to inversions that caused deleterious growth in *E. coli* (REBOLLO *et al.* 1988). We did not observe any severe growth defect in the strains carrying these inversions, and we saw no evidence in the *XbaI* digestion patterns that the strains accumulated secondary rearrangements. The cause of these differences between *S. typhimurium* and *E. coli* is not obvious.

Thirty-four large inversions originating from five different *Tn10*s at four chromosomal locations were characterized in these studies. These inversions fell into two general size classes. Because of the genetic criterion in the isolation of these strains, the inversions were expected to be larger than ~45 kb. Of the characterized inversions, 16/34 (47%) were 35–85 kb in size. These relatively small inversions were expected based on the preference of *Tn10* transposase for acting on "nearby"

DNA. Among these relatively short inversions, there was no preference for the clockwise (6/12) or counterclockwise (6/12) direction in the *aroD::Tn10* and *pyrA::Tn10* inversions, although the *trp::Tn10* inversions were all (4/4) counterclockwise of the *trp* insertion site.

The very large inversions document a bias in transposase selection of distant inversion endpoints. These endpoints were not chosen at random, which would have resulted in a scattering of distant endpoint locations. Rather, each insertion had a preferred inversion distant endpoint region (of ~100–200 kb) that was selected at least twice as often as all other distant locations combined. These results are consistent with the transposase search in a limited volume for a distant target, and an organized, relatively inflexible folded bacterial nucleoid *in vivo*.

The remaining inversions endpoints which lay in non-preferred regions may reflect a hierarchy of preference for the remaining chromosomal regions, or may show a random scatter of the non-preferred endpoints. Distinguishing between these possibilities must await more data.

There was some preference for the inversion endpoints to end within the *XbaI* "A" and "C" bands. However, since some insertions did not yield these inversion endpoints (e.g., the *purD877::Tn10*-derived inversions ended within the *XbaI* "E" band), and since the *pyrA*, *trp* and *aroD* inversions targeted different locations within the *XbaI* "A"

and "C" bands (see Figure 4), we believe the apparent preference for these two regions is coincidental.

The large inversions had a high incidence of additional *IS10* transposition events. These new *IS10* insertions document additional distant target sites for the transposase. However, since the *IS10*-transposase complex may have a "free" intermediate state (BENJAMIN and KLECKNER 1992), Tn10-mediated inversion and *IS10* transposition may have different constraints. Tn10-mediated inversion must result in the ligation of linearly distant chromosomal sequences, an event which requires these chromosomal DNAs to at least temporarily be in extremely close physical proximity.

The data presented here and the extensive knowledge of Tn10 transposase reactions combined to eliminate some potential explanations for preferred distant inversion endpoint regions. First, although the distant inversion endpoints clustered, the inversions were not identical, since the novel bands differed in size. Thus, while the *IS10* transposase is known to prefer certain "hotspot" sequences, which can be influenced by the sequence context (BENDER and KLECKNER 1992; NOEL and AMES 1978; KLECKNER *et al.* 1979), the preferred inversion endpoint regions are a different phenomenon.

The distant target region preferred by one Tn10 insertion is not preferred by all insertions. Because of this, the correct explanation must require a proper "match" between the donor and target sites. Thus, explanations for the preferred target regions that are based on the amounts of bound HU, IHF, or H-NS proteins, or on the level of DNA supercoiling, would require the unique combination of their amounts at the donor and target regions to explain the data. Similarly, we find the relatively infrequent transposition of Tn10 into transcriptionally active regions (CASADESUS and ROTH 1989) to be an unlikely explanation. Transcriptionally quiet chromosomal regions have not been identified in *E. coli* (CHUANG *et al.* 1993). Nor is there currently any evidence of transient transcriptional inactivity of chromosomal regions during the replication cycle of *E. coli* or *S. typhimurium*.

What hypotheses can be entertained to explain these data? Three general conditions must exist in order to recover strains carrying the products of an illegitimate recombination. The DNA sequences must interact, the enzymological events must occur, and the resulting strains must be viable. In the case of Tn10-mediated inversions, the enzymological events are relatively well defined and provide no clues to the source of the preferred inversion targets. The viability of strains carrying a wide variety of large inversions has been shown (REBOLLO *et al.* 1988; SEGALL *et al.* 1988). To initiate the illegitimate exchanges, the transposase-bound *IS10* sequences must physically contact the distant target sequence in such a manner that productive enzymatic events occur. The very large inversions generated by Tn10 may sample the most frequent DNA-DNA inter-

actions that have the correct spatial orientation and position, during the short time window when the transposase is active.

The spatial proximity of non-contiguous DNA sequences can be affected by the DNA's topology. The probability of DNA strand-strand interaction is increased about 60-fold by plectonemical interwinding of DNA (KLENIN *et al.* 1991; RYBENKOV *et al.* 1993). The 5 Mbp *S. typhimurium* or *E. coli* DNA genome is thought to be organized into about 40 nucleoid domains, which would make the average domain size ~120-kb (SINDEN and PETTJOHN 1981; WORCEL and BURGI 1972). Plectonemical interwinding might be a predominant feature of a single nucleoid domain, which could lead to the preference for transposase interaction 40–80 kb on either side of the insertion site. In addition, the two double strands of the plectonemically interwound DNA will have oppositely oriented helix axes, which might provide the proper spatial orientation for inversion. Cat- enation of circular DNA molecules can also increase the likelihood that sites present on different circular molecules will contact one another. Analogously, nucleoid structure might constrain the configuration of different DNA domains, causing certain distant DNA donor and target regions to have more frequent interactions.

Using this interpretation, the data provide a point-by-point assessment of which DNAs are spatial neighbors of one another in the bacterial nucleoid. Several of these inversions predict spatial proximity of DNAs that are equidistant from the origin of DNA replication. The preferred distant target region of *trp::Tn10*-catalyzed inversions was centered around *Ter*, while one of the very large *pyrA* inversions was centered around *OriC* (see Figure 4). Since the transposase is active just after DNA replication of the insertion has occurred, these data suggest that the oppositely directed replication forks, proceeding at similar speeds, are spatially near one another in the cell.

With the limited available data, additional spatial associations are predicted, but the underlying pattern is not yet apparent. The current data may indicate that different chromosomal locations have different flexibilities within the folded structure. While insertions in the *purD* locus preferentially chose one relatively small chromosomal region as an inversion target, insertions in the *pyrA* locus target several distant chromosomal locations.

The results show a highly non-random pattern of Tn10-catalyzed large inversions, that is best explained by the preferential action of the Tn10 transposase within a local three-dimensional realm. These results may provide an *in vivo* method for determining the folding pattern of DNA in the bacterial nucleoid.

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