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Tn*7* Transposition as a Probe of *cis* Interactions between Widely Separated (190 Kilobases Apart) DNA Sites in the *Escherichia coli* Chromosome

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We have used the bacterial transposon Tn*7* to examine communication between widely separated DNA sites in the *Escherichia coli* chromosome. Using Tn*7* target immunity, a regulatory feature of transposition which influences target selection, we have evaluated (i) how the presence of Tn*7* sequences at one DNA site affects Tn*7* insertion into another site in the same DNA molecule and (ii) the nucleotide distances over which the two sites are able to communicate. We demonstrate that Tn*7* sequences at one chromosomal site act at a distance to inhibit insertion of Tn*7* elsewhere in that DNA as far away as 190 kb, reflecting effective long-range *cis* interactions. We have found that while target immunity is effective over a substantial region of the chromosome, insertion of Tn*7* into a more distant site 1.9 Mb away in the same DNA is not inhibited; this observation provides evidence that target immunity relies on DNA spacing. We also find that within the region of the chromosome affected by target immunity, the magnitude of the immune effect is greater at close DNA sites than DNA sites farther away, suggesting that target immunity is distance dependent. We also extend the characterization of the Tn*7* end-sequences involved in transposition and target immunity and describe how Tn*7* target immunity can be used as a tool for probing bacterial chromosome structure.

Interactions between protein-DNA complexes at different sites in the same DNA molecule underlie many biological processes, including DNA replication, transcription, and recombination. Moreover, many of these interactions entail communication between DNA sites that are separated by nucleotide distances of hundreds or thousands of base pairs. It is interesting to know over what nucleotide distances such interactions can be effective. We are using transposition of Tn*7* (8, 11), a bacterial transposon, as a tool to probe the degree of interaction between widely separated DNA sites in the *Escherichia coli* chromosome.

Tn*7* is one of several transposable elements regulated by a long-range feature of the target DNA called transposition immunity or target immunity. Target immunity is the ability of a transposon already occupying a DNA molecule to substantially reduce the frequency of a subsequent transposon insertion into that DNA (27). Transposition into other DNA molecules elsewhere in the cell is not inhibited, so that target immunity is a *cis* effect and not a global, *trans*-inhibition. Thus, target immunity is a negative feature of the target DNA.

Tn*7* also responds positively to certain features of the target DNA. Depending on which target signals are present, two overlapping sets of Tn*7*-encoded proteins can mediate transposition into two different classes of target sites (20, 28, 33). In one transposition pathway, the Tn*7* proteins TnsABC+D direct insertion into a unique site in the *E. coli* chromosome called *attTn7* (8, 22) through specific recognition of sequences in *attTn7* (7, 34). In the other transposition pathway, the Tn*7* proteins TnsABC+E ignore the *attTn7* target and instead direct insertion into non-*attTn7* sites. Furthermore, the TnsABC+E pathway preferentially directs Tn*7* transposition into bacterial plasmids that can conjugate between cells, and the resulting insertions occur at many different plasmid sites (36). Although Tn*7* target selection in the two transposition pathways is strikingly different, previous studies have shown that target immunity can work in both of these transposition pathways (5).

Analysis of the target immunity phenomenon using plasmid targets demonstrated that the sequences at the transposon ends which are the substrate for transposition are responsible for the immune effect (2, 12, 16, 21). In the case of Tn*7* (5), the frequency of transposon insertion into a plasmid target is reduced substantially when the plasmid already contains either full-length Tn*7* or a mini-Tn*7* element that contains only the transposon end-sequences flanking an antibiotic resistance gene. Although Tn*7* can usually insert into many different sites in the pOX38-Gen plasmid (36), a derivative of the F plasmid (18), the presence of a mini-Tn*7* element at any position in pOX38-Gen substantially inhibits Tn*7* insertion into any other site in the 65-kb plasmid. Therefore, the presence of transposon ends at one DNA site can influence transposon insertion into another site in the same DNA molecule.

What features of the target DNA mediate communication between the transposon ends at one site and potential target sites elsewhere in that immune target DNA? An attractive view is that DNA looping in the target DNA facilitates contacts between these sites. That is, the DNA backbone itself is a feature of the target DNA which increases the frequency of collisions between DNA sites at different positions, promoting *cis*-interactions. Such a role for the DNA backbone is supported by in vitro findings for transposition of bacteriophage Mu which is also regulated by target immunity: Mu end-sequences in one plasmid can confer immunity to another plasmid lacking Mu end-sequences when the two DNA molecules are catenated (3). Thus, a reasonable model is that DNA spacing between the transposon end-sequences and a potential

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insertion site elsewhere in that target DNA can influence the magnitude of the target immunity because contacts between DNA sites are expected to be more frequent over shorter distances. However, the contribution of DNA spacing has not actually been examined for any of the transposable elements that are known to be regulated by target immunity: bacteriophage Mu, Tn*3*, and Tn*7*. Alternatively, it is not unreasonable to consider that target immunity may be influenced by features of the target DNA besides DNA spacing. One plausible model is that immunity is imposed by a mechanism involving sequestration by compartmentation that renders every site in a target DNA equally immune to insertion, independent of DNA spacing.

To more directly examine the contribution of DNA spacing, it is important to evaluate simultaneously the amount of transposon insertion into at least two target sites in the same DNA: one target site relatively near the transposon ends that provide immunity and another target site relatively far away. It is also interesting to examine the immune effect in the bacterial chromosome where successive transposon insertions pose a mutagenic threat to the host cell; target immunity has not previously been examined in the bacterial chromosome. In this report, we demonstrate that target immunity can substantially inhibit Tn*7* insertion into a relatively near target site in the *E. coli* chromosome without affecting insertion into a second target site, 1.9 Mb away in the same DNA, supporting the view that target immunity relies on DNA spacing for these *cis*-interactions which are effective at 190 kb.

We have used the *E. coli* chromosome as a transposition target to evaluate how the presence of Tn*7* sequences at one chromosomal site affects site-specific Tn*7* insertion elsewhere in the chromosome. Tn*7* can preferentially transpose at high frequency and in one orientation into a unique site in the *E. coli* chromosome called *attTn7* (8, 22). Tn*7* transposition into *attTn7* can be measured directly by a Southern blot analysis. To evaluate the target immunity effect, a mini-Tn*7* element was positioned at a particular chromosomal site outside *attTn7*, and subsequent Tn*7* insertion into *attTn7* was measured. The degree to which the mini-Tn*7* element can inhibit subsequent Tn*7* insertion indicates the level of interaction between the two chromosomal sites. By changing the DNA spacing between the position of the mini-Tn*7* element and *attTn7*, the range of DNA over which two chromosomal sites can effectively interact was evaluated. In this report, we demonstrate that the long-range DNA interactions necessary for target immunity are alleviated by distance and can be effective at least 190 kb away in the *E. coli* chromosome.

MATERIALS AND METHODS

Chromosomal gene replacements. One- to 2-kb segments of the appropriate nonessential host genes (see below) were appended to both ends of the DNA inserts *attTn7-cat*, mini-Tn*7*, and a kanamycin resistance gene (*kan*), enabling homologous recombination into precise positions of the *E. coli* chromosome. DNA inserts flanked by host sequences were electroporated into a *recD* strain, DPB271 (29), and homologous recombinants were identified by direct antibiotic selection and Southern blot analysis. Each recombinant chromosomal site was moved by phage P1 transduction to a standard strain for Tn*7* transposition, NLC 28 (MC4100 Rec^+ Val^r). The identification of suitable host genes was aided by the program GeneScape 0.9 (9) and the data set EcoSeq6/EcoMap6/EcoGene6 provided by Kenneth Rudd at the National Center for Biotechnology Information.

Position of $attTn7_{44}$. A 507-bp segment of the chromosomal *attTn7* site (−342 to +164) was joined to a chloramphenicol resistance gene (*cat*), such that position +164 of *attTn7* abuts the *cat* promoter. A fragment containing the *attTn7-cat* sequences was introduced between nucleotides 128 and 140 of the *hisC* gene open reading frame (ORF).

Positions of mini-Tn*7* or *kan*. The *kan* gene or mini-Tn*7* (with *kan* cloned between the left and right Tn*7* ends) was introduced into four *E. coli* chromosome genes: between nucleotides 511 and 564 of the *hisH* gene ORF, 3 kb from $attTn7_{44}$; between nucleotides 379 and 454 of the *xylB* gene ORF, 190 kb from $attTn7_{84}$; between nucleotides 921 and 922 of the *ilvB* gene ORF, 64 kb from $attTn7_{84}$; and between nucleotide 1741 of the *bglS* gene ORF and nucleotide 205 of the *bglB* gene ORF, 8 kb from $attTn7_{84}$. The immobile mini-Tn*7* element was isolated from pSIM (14), and *kan* was isolated from pUC4K (Pharmacia). A plasmid containing *ilvB* sequences, pYYC37, was provided by Ying-Ying Chang and John Cronan at the University of Illinois.

Southern blot analysis and quantitation. Independent bacterial colonies were grown overnight on LB plates and expanded to saturation in 5-ml LB cultures before isolation of genomic DNA as described previously (35). DNA was digested with *Eco*RV, separated on 0.6% agarose gels, denatured, and transferred to GeneScreen Plus (NEN Research Products). Blots were hybridized either with a *glmS* probe comprised of sequences near (+177 to +1148) $attTn7_{84}$ or with a *cat* probe, the CAT GenBlock (Pharmacia), containing sequences that were adjoined to $attTn7_{44}$. Tn*7* insertion was quantified by using phosphorimager analysis in the linear range of detection (pixel values < 100,000). Two signals in each gel lane were measured by volume integration using ImageQuant version 3.3 (Molecular Dynamics): empty *attTn7* and insertion in *attTn7*. To quantify background, the nonspecific hybridization of probe to genomic DNA, two signals were measured in gel lanes containing DNA from cells without any Tn*7*: the same electrophoretic position and area used to measure insertion in *attTn7* and empty *attTn7*. We found that background contributes significantly to the Tn*7* insertion signal in strains with strong target immunity effects, although the contribution is small in strains without target immunity effects. To focus on the substantial differences in the magnitude of target immunity at each chromosomal position rather than the minor fluctuations that occur between clonal populations, relative Tn*7* insertion was calculated by using raw, uncorrected data without subtracting background; the contribution of background to Tn*7* insertion signal is displayed directly on the histograms of relative Tn*7* insertion.

Mating-out analysis. Transposition of Tn*7* from the chromosome into a conjugative plasmid, pOX38-Gen (18), was done as described previously (33) except that mating mixtures were plated on LB agar with 10 μg of nalidixic acid, 50 μg of rifampin, and 7.5 μg of gentamicin per ml to select all exconjugants and plated on Iso-Sensitest agar (Oxoid) with 10 μg of nalidixic acid, 50 μg of rifampin, and 50 μg of trimethoprim per ml to select only exconjugants with a Tn*7* insertion. Transposition frequency is expressed as the fraction of exconjugants that obtain a transposon insertion. Transposition of a mobile mini-Tn*7* element comprised of a *kan* gene flanked by wild-type Tn*7* ends, supported by Tn*7*-encoded transposition proteins supplied in *trans* from pCW4 (33), was detected by plating mating mixtures on LB agar with 10 μg of nalidixic acid and 50 μg of kanamycin per ml.

Bacteria. The general bacterial methods of transformation, phage P1 transduction, and plasmid conjugation were done as described previously (24).

RESULTS

Direct detection of Tn*7* insertion into *attTn7* in the *E. coli* chromosome. Tn*7* can transpose preferentially into a unique site at min 84 in the *E. coli* chromosome called *attTn7*, inserting about 25 bp downstream of a bacterial gene, *glmS*, that encodes a protein involved in cell wall biosynthesis (15, 22). A Southern blot of genomic DNA probed with the chromosomal *glmS* sequence can distinguish between cells with a Tn*7* insertion in *attTn7* (5.5 kb) and cells without a Tn*7* insertion in *attTn7* (2.8 kb) (Fig. 1, lanes 1 and 2).

A Southern blot of clonal populations active for Tn*7* transposition reveals a high frequency of insertion into the *attTn7* site. These clonal populations are made by introducing a plasmid containing Tn*7* into a cell lacking Tn*7*. Colonies are grown out from single cells (>25 generations) in the absence of explicit selection and expanded to saturation (<10 generations) in small culture volumes. During outgrowth from a single cell, Tn*7* transposes from the plasmid to the chromosome in individual cells and the population accumulates Tn*7* insertions in the *attTn7* site. Southern analysis of genomic DNA from six clonal populations (Fig. 1, lanes 3 to 8) shows that a considerable fraction (about 2%) of progeny obtain a Tn*7* insertion, reflecting the high frequency of Tn*7* transposition into the *attTn7* site in the *E. coli* chromosome. This level of Tn*7* insertion is representative of most populations, but there are occasional (approximately 1 in 30) populations of "jackpots" in which the level of Tn*7* insertion appears much higher because a transposition event occurred at an earlier than usual time of colony growth (data not shown).

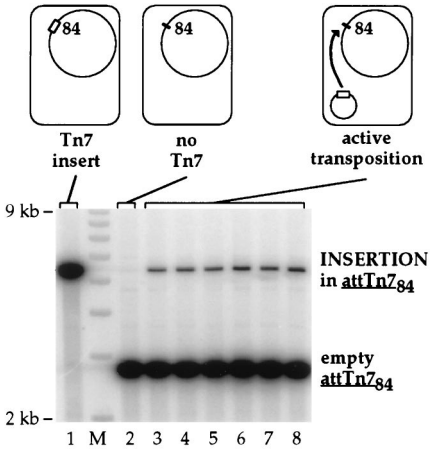

FIG. 1. Southern blot analysis of Tn*7* insertion into the chromosomal *attTn7* site, using the *glmS* probe. Lanes 3 to 8 are independent populations grown from single cells that are active for transposition of Tn*7* (rectangle) from a donor plasmid (small circle) to the *attTn7* site (at min 84) in the *E. coli* chromosome (large circle). Lanes 1 and 2 are pure populations of cells with and without Tn*7* in the *attTn7* site, respectively, and lane M is a 1-kb ladder.

Simultaneous detection of Tn*7* insertion into two chromosomal sites. Our strategy to probe target immunity in the chromosome was to place Tn*7* ends at different positions in the chromosome and evaluate their effects on insertion into *attTn7* at min 84, *attTn7*$_{84}$. To also establish that any transposition inhibition observed in the cell was authentic target immunity, a local phenomenon rather than global, we also evaluated the effects of the Tn*7* ends on a distant insertion site. Thus, a second copy of *attTn7* was introduced into the chromosome 1.9 Mb away from *attTn7*$_{84}$ by gene replacement of the chromosomal *hisC* gene at min 44, generating *attTn7*$_{44}$. To enable selection during gene replacement and provide unique sequences for Southern blot hybridization, the *attTn7* segment was adjoined to *cat*. A Southern blot of genomic DNA probed with the *cat* sequence can distinguish between cells with (5.5 kb) or without (1.5 kb) a Tn*7* insertion in the new chromosomal target site.

In a clonal population, Tn*7* transposition from a plasmid to both *attTn7*$_{84}$ and *attTn7*$_{44}$ can be measured simultaneously by analyzing chromosomal DNA on two Southern blots, using a *glmS* probe to detect Tn*7* insertion in *attTn7*$_{84}$ and a *cat* probe to detect Tn*7* insertion in *attTn7*$_{44}$. We found the new (*attTn7*$_{44}$) chromosomal target site used for Tn*7* insertion is active at a level comparable (5%) to that for the endogenous (*attTn7*$_{84}$) insertion site (Fig. 2A and 2B, lanes 1 to 3). We also found that the new chromosomal target site is active when the *attTn7* sequences at min 44 are inverted within the *hisC* gene (data not shown), establishing that the 507-bp segment used to generate *attTn7*$_{44}$ is sufficient for directing high-frequency, site-specific Tn*7* insertion.

Target immunity acts locally in the *E. coli* chromosome. Having two *attTn7* insertion sites in the same chromosome allows us to investigate how a Tn*7* element already residing in the chromosome affects subsequent Tn*7* insertion into that chromosome both locally and globally. A mini-Tn*7* element comprised of 166 bp of the left end and 199 bp of the right end of Tn*7* is sufficient to provide target immunity when present in a plasmid DNA (5). To fix the position of the otherwise mobile Tn*7* end-sequences at a particular chromosomal site near one of the two *attTn7* sites, a mini-Tn*7* element immobilized by mutation of the terminal dinucleotides in the left and right transposon ends (14) was introduced into the chromosome by gene replacement. We positioned mini-Tn*7* relatively closer to *attTn7*$_{44}$ and evaluated Tn*7* insertion into both the nearby *attTn7*$_{44}$ site and the *attTn7*$_{84}$ site 1.9 Mb away. We have found that target immunity inhibits subsequent Tn*7* insertion into the chromosome only in the vicinity of the mini-Tn*7* element.

A Southern blot analysis of cells with mini-Tn*7* at 3 kb from *attTn7*$_{44}$ reveals that target immunity inhibits Tn*7* insertion into the nearby *attTn7*$_{44}$ (Fig. 2B, lanes 5 to 7). Due to the profound immune effect, the insertion signal in *attTn7*$_{44}$ is nearly as low as background, which confounds an exact measurement of the degree of inhibition; however, a reasonable estimate is that target immunity has at least a 12-fold effect at this very close nucleotide distance. In contrast, insertion into the distant *attTn7*$_{84}$ in the same cells is not inhibited (Fig. 2A, lanes 5 to 7). Thus, this mini-Tn*7* has no effect on Tn*7* insertion into *attTn7*$_{84}$, 1.9 Mb away, revealing that mini-Tn*7* in the chromosome inhibits insertions only locally, not globally. (We show below that target immunity is active on *attTn7*$_{84}$ when a mini-Tn*7* is positioned relatively nearby.) Therefore, target immunity in the chromosome is a local effect that is sensitive to the position of the mini-Tn*7* transposon ends relative to potential insertion sites within the same DNA molecule.

The mini-Tn*7* element which can provide target immunity in the chromosome contains both the Tn*7* end-sequences that

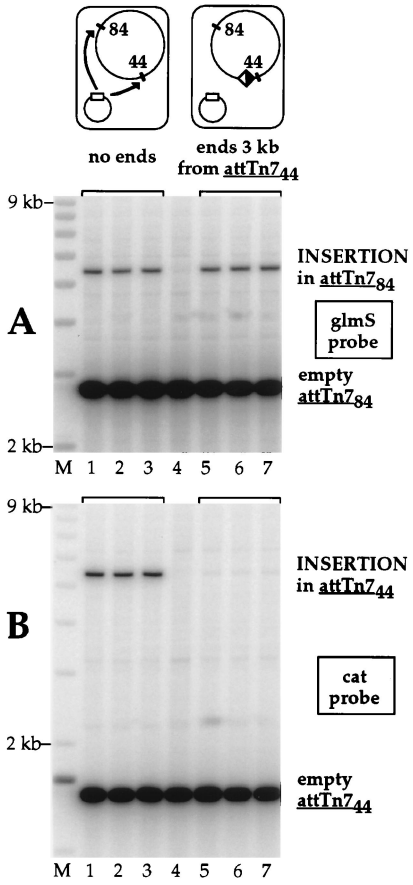

FIG. 2. Southern blot analysis to simultaneously score Tn*7* insertion into both *attTn7*$_{84}$ (A; *glmS* probe) and *attTn7*$_{44}$ (B; *cat* probe), using cells without a mini-Tn*7* element in the chromosome (lanes 1 to 3) or cells with a mini-Tn*7* element (shaded diamond; comprised of the Tn*7* end-sequences flanking a *kan* gene) in the chromosome positioned near *attTn7*$_{44}$ (lanes 5 to 7). Lane 4 displays a pure population of cells lacking Tn*7*, and lane M is a 1-kb ladder.

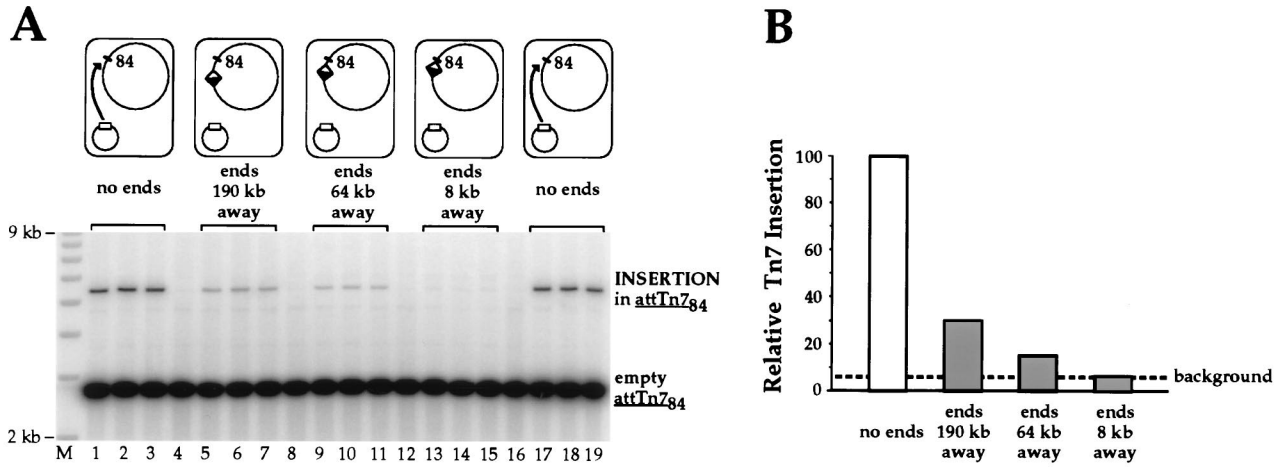

FIG. 3. Southern blot analysis (A) and quantitation (B) of Tn*7* insertion into *attTn7*$_{84}$, using cells without a mini-Tn*7* element in the chromosome (lanes 1 to 3 and 17 to 19, $n = 6$) or cells with a mini-Tn*7* element (shaded diamond) in the chromosome positioned either 190 kb (lanes 5 to 7, $n = 3$), 64 kb (lanes 9 to 11, $n = 3$), or 8 kb (lanes 13 to 15, $n = 3$) away from *attTn7*$_{84}$. Lanes 4, 8, 12, and 16 display pure populations of cells lacking Tn*7*, and lane M is a 1-kb ladder. The mean fraction of insertion for n populations is expressed relative to Tn*7* insertion in cells without a mini-Tn*7* element (100 = 2.3% occupancy, which is the mean of lanes 1 to 3 and 17 to 19). The contribution of background hybridization to the Tn*7* insertion signal in cells without a mini-Tn*7* element was determined (Materials and Methods) from the four cell populations lacking Tn*7* and is displayed as a dashed line (*attTn7*$_{84}$ background = 8).

participate in transposition and *kan*. We have established that target immunity depends only on the presence of the Tn*7* end-sequences in the chromosome, not *kan*: simple gene disruptions containing only *kan* do not affect the level of Tn*7* insertion into a nearby *attTn7* site (data not shown).

The left and right ends of Tn*7* and of mini-Tn*7* are functionally and structurally distinct. The ends differ, for example, in the number and arrangement of the essential transposition protein binding sites (5, 11). Do the asymmetric ends of Tn*7* affect target immunity differently? We examined how the orientation of the mini-Tn*7* end-sequences relative to the insertion site in the chromosome may affect target immunity and found that Tn*7* insertion into *attTn7*$_{84}$ or *attTn7*$_{44}$ is inhibited to about the same degree when a mini-Tn*7* element in either orientation is positioned nearby (data not shown), indicating that target immunity for Tn*7* is not a unidirectional effect. Therefore, target immunity appears to be constrained to a local region of the *E. coli* chromosome and operates in both directions from the mini-Tn*7*.

Target immunity is effective over at least 190 kb. We showed above that a mini-Tn*7* element in the *E. coli* chromosome provides target immunity in a local fashion, inhibiting subsequent Tn*7* insertion into a relatively close (3 kb away) *attTn7* site without affecting insertion into a relatively distant site, 1.9 Mb away. To assess the size of the local region affected by target immunity, Tn*7* insertion into *attTn7*$_{84}$ was evaluated in cells with a mini-Tn*7* element positioned 8, 64, or 190 kb away from *attTn7*$_{84}$.

A Southern blot analysis reveals the striking nucleotide distances at which we found target immunity is effective in the chromosome. Even in cells with a mini-Tn*7* element positioned 190 kb from *attTn7*$_{84}$ (Fig. 3, lanes 5 to 7), Tn*7* insertion is inhibited at least threefold compared with the level of Tn*7* insertion in cells without a mini-Tn*7* element in the chromosome (Fig. 3, lanes 1 to 3 and 17 to 19). Therefore, the local region of the chromosome affected by target immunity can extend at least 190 kb from the mini-Tn*7* element. As described above, target immunity operates in both directions from mini-Tn*7*, and so a substantial fraction [8% $\approx$ (190 kb + 190 kb)/4,700 kb] of the *E. coli* chromosome can be influenced by the presence of the Tn*7* end-sequences.

Within the local immune region of the chromosome, we observe different degrees of target immunity at different distances, with a higher degree of inhibition at shorter nucleotide distances. Compared with the approximately 3-fold inhibition of Tn*7* insertion in cells with a mini-Tn*7* element positioned 190 kb from *attTn7*$_{84}$ (Fig. 3, lanes 5 to 7), the target immunity effect is about 6-fold when the mini-Tn*7* element is positioned 64 kb from *attTn7*$_{84}$ (Fig. 3, lanes 9 to 11) and greater than 12-fold when it is positioned 8 kb from *attTn7*$_{84}$ (Fig. 3, lanes 13 to 15).

The nucleotide distance in the chromosome affected by target immunity is striking. To confirm that effects at these considerable distances actually represent immunity and not a global inhibition of transposition, we analyzed how the mini-Tn*7* at these chromosomal positions affects Tn*7* insertion in cells with two *attTn7* sites. Southern analysis reveals that mini-Tn*7* at either location affects Tn*7* insertion at the local *attTn7* site but not the distal site. Positioning the mini-Tn*7* element either 64 or 190 kb from *attTn7*$_{84}$ provides target immunity to the nearby *attTn7*$_{84}$ (Fig. 4A), albeit with a lesser degree of inhibition than observed when mini-Tn*7* was positioned 3 kb from *attTn7*$_{44}$ or 8 kb from *attTn7*$_{84}$ (Fig. 2 and 3). While the inhibition is effective as far away as 190 kb, mini-Tn*7* at either position near *attTn7*$_{84}$ acts only locally and does not affect Tn*7* insertion into *attTn7*$_{44}$, 1.9 Mb away within the same cells (Fig. 4B). Therefore, target immunity can affect a substantial region of the *E. coli* chromosome and is alleviated when DNA spacing is increased.

The results presented above support the view that DNA spacing between the transposon ends at one site and a potential insertion site elsewhere in the transposition target determines the degree of the target immunity effect. That is, closer nucleotide distances have a stronger target immunity effect than farther nucleotide distances. We cannot, however, exclude the possibility that the target immunity effect observed with a mini-Tn*7* at a particular chromosomal position is influenced not only by nucleotide distance from the potential insertion site but also by chromosomal context. That is, a greater degree of target immunity might be observed in the absence of some other chromosomal process such as transcription. Transcription could, for example, interfere with target immunity by

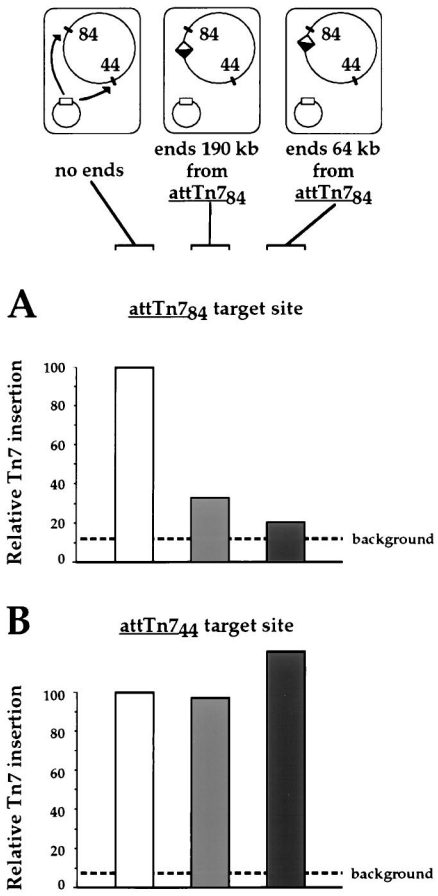

FIG. 4. Quantitation of Tn*7* insertion into *attTn7*$_{84}$ (A) and *attTn7*$_{44}$ (B), using Southern blot analysis for cells with ($n = 3$) or without ($n = 6$) a mini-Tn*7* element near *attTn7*$_{84}$. The mean fraction of insertion for n populations is expressed relative to Tn*7* insertion in cells without a mini-Tn*7* element (A, 100 = 2.0% occupancy; B, 100 = 5.5% occupancy). The contribution of background hybridization to the Tn*7* insertion signal in cells without a mini-Tn*7* element (set at 100) was determined (Materials and Methods) from four cell populations lacking Tn*7* (data not shown) and is displayed as a dashed line (*attTn7*$_{84}$ background = 12; *attTn7*$_{44}$ background = 8).

encroaching upon the end-sequences of mini-Tn*7*, although we do not believe that these mini-Tn*7* elements have been positioned within highly transcribed regions of the chromosome. We have observed, however, that the effectiveness of target immunity appears to be influenced qualitatively by the length of the chromosomal DNA backbone connecting the *attTn7* site and the mini-Tn*7* element: the immune effect is very strong at close distances (3 or 8 kb) and intermediate in strength at farther distances (64 or 190 kb) but not detectable at very long distances (1.9 Mb).

Immunity acts exclusively on target DNA. A mini-Tn*7* element located in a target DNA can act over a considerable distance to inhibit Tn*7* insertion. Does mini-Tn*7* affect transposition when located at the same kinds of distances from Tn*7* in the donor DNA? We examined how end-sequences positioned 64 kb away from an *attTn7*$_{84}$::Tn*7* donor site affect transposition of Tn*7* from *attTn7*$_{84}$ to pOX38-Gen. Although a nucleotide distance of 64 kb from the insertion site is sufficiently close for end-sequences in a target DNA to effectively inhibit Tn*7* insertion (Fig. 3), the presence of end-sequences at the same distance from the donor site does not alter Tn*7* transposition: the frequency of Tn*7* transposition from

TABLE 1. Effects of mini-Tn*7* location on Tn*7* transposition[a]

Mini-Tn*7* location	Mean Tn*7* transposition frequency (%) ± SD[b]
None	100 ± 28 (11)
In donor	102 ± 58 (12)
In target	2 ± 2 (8)

[a] Mating-out analysis of Tn*7* transposition from *attTn7*$_{84}$ in the chromosome to a pOX38-Gen plasmid, using cells without a mini-Tn*7* element (none) or cells with a mini-Tn*7* element located either in the chromosome 64 kb from the *attTn7*$_{84}$ donor site (in donor) or in pOX38-Gen (in target).

[b] The mean transposition frequencies for n (in parentheses) populations are expressed as percentages of Tn*7* transposition in cells without a mini-Tn*7* element. 100% = a mean of 3.9×10^{-5}.

attTn7$_{84}$ in the chromosome to pOX38-Gen appears the same in strains with or without a mini-Tn*7* element positioned 64 kb from the *attTn7*$_{84}$ donor site (Table 1). Therefore, immunity acts exclusively on target DNA, and is not a global inhibition of transposition.

Transposition sequences within the Tn*7* ends are sufficient to provide immunity. Previous work established that a mini-Tn*7* element comprised of 166 bp of the left end (Tn*7*L) and 199 bp of the right end of Tn*7* (Tn*7*R) can effectively provide target immunity when present in a target plasmid (5). To further define which sequences in the Tn*7* ends are necessary for target immunity, we evaluated how shortening Tn*7*R to 90 bp affects the ability of mini-Tn*7* to provide target immunity. A mini-Tn*7* element comprised of 166 bp of Tn*7*L and 90 bp of Tn*7*R (L166-R90) contains all sequences necessary for efficient transposition (Table 2); most notable in these DNA segments are the multiple binding sites for TnsB, the Tn*7*-encoded protein that is required for all Tn*7* transposition reactions (4, 6, 7, 23). There are three separate binding sites for TnsB in Tn*7*L and four contiguous sites in Tn*7*R.

A mini-Tn*7* element containing 90 bp of Tn*7*R can effectively provide target immunity. When Tn*7* transposes from the *E. coli* chromosome to a plasmid, the frequency of transposition is inhibited as much as 100-fold when the target plasmid, pOX38-Gen, contains a mini-Tn*7* element (Table 3). The level of inhibition is not diminished when internal transposon sequences beyond 90 bp of Tn*7*R are removed (L166-R199, L166-R141, and L166-R90) (5), indicating that target immunity requires only the end-sequences containing the TnsB binding sites that are necessary for transposition. Indeed, deletion of a TnsB binding site in Tn*7*R (L166-R70) reduces the ability of mini-Tn*7* both to transpose and to provide target immunity (Tables 2 and 3), as was noted previously (5).

We note that while the Tn*7* end-sequences that are necessary for transposition are involved in target immunity, mobilization of the end-sequences is not required. In bacteriophage

TABLE 2. End-sequences required in transposition[a]

Mini-Tn*7*	Mean mini-Tn*7* transposition frequency (%) ± SD[b]
L166-R199	100 ± 27
L166-R142	93 ± 25
L166-R90	130 ± 40
L166-R70	16 ± 6

[a] Mating-out analysis of mini-Tn*7* elements with right ends of different lengths transposing from *attTn7*$_{84}$ in the chromosome to the pOX38-Gen plasmid.

[b] The mean transposition frequencies for 12 populations in each case are expressed as percentages of mini-Tn*7* L166-R199 transposition. 100% = a mean of 3.3×10^{-5}.

TABLE 3. End-sequences involved in target immunity[a]

Target plasmid	Mean Tn*7* transposition frequency (%) ± SD[b]
pOX38-Gen	100 ± 61 (12)
pOX38-Gen::L166-R199	0.3 ± 0.2 (10)
pOX38-Gen::L166-R142	0.5 ± 0.5 (12)
pOX38-Gen::L166-R90	0.4 ± 0.4 (12)
pOX38-Gen::L166-R70	24 ± 12 (12)

[a] Mating-out analysis of Tn*7* transposition from *attTn7*$_{84}$ in the chromosome to a pOX38-Gen plasmid with or without a mini-Tn*7* element.

[b] The mean transposition frequencies for *n* (in parentheses) populations are expressed as percentages of Tn*7* transposition to pOX38-Gen (without a mini-Tn*7*). 100% = a mean of 4.7×10^{-5}.

Mu and Tn*3* transposition, the transposon termini that are essential for mobilization are not necessary for target immunity; the transposase binding sites within the end-sequences are sufficient to confer target immunity (12, 21). The chromosomal immunity experiments in this study used a mini-Tn*7* element which is immobilized by a 2-bp mutation at the transposon termini that are the sites of strand breakage and joining during transposition (14); we have shown here the immobile mini-Tn*7* element when present in a target DNA can effectively provide target immunity (Fig. 2 to 4). Therefore, mobilization of the end-sequences is not necessary for target immunity. An attractive model is that the TnsB binding sites within the transposon ends that are the substrate for transposition are the sequences that mediate Tn*7* target immunity (32).

DISCUSSION

Tn*7* target immunity is a striking example of action at a distance: we have found that a Tn*7* element in the *E. coli* chromosome can act over a distance of 190 kb to inhibit insertion of a second Tn*7* element into that DNA. As discussed below, Tn*7* target immunity in the chromosome likely involves protein-protein interactions between a nucleoprotein complex at the Tn*7* sequences that provide target immunity and the nucleoprotein complex at the *attTn7* target site in that same DNA. How do these two protein-DNA complexes find each other when the DNA sites are widely separated, as far apart as 190 kb? DNA looping is a model often invoked to explain how DNA sites separated by hundreds or thousands of base pairs communicate with each other (1); the number of collisions between distant DNA sites is influenced by the length of the DNA backbone (30). Moreover, the *E. coli* chromosome is compacted nearly 1,000-fold in living cells; DNA supercoiling and packaging by histone-like proteins may effect condensation of larger nucleotide distances in the chromosome (26). In this study, we have found that target immunity in the chromosome appears consistent with the DNA backbone effecting communication between widely separated DNA sites: target immunity works only in *cis*, within the same DNA, and is more effective at shorter nucleotide distances than longer, establishing a local, albeit extensive region of target immunity in the chromosome. Our experiments suggest that effective *cis*-interactions that are influenced by nucleotide distance can occur over a considerable portion of the chromosome; accordingly, 190 kb is a relatively local distance in the *E. coli* chromosome.

How do the transposon end-sequences act at a distance in a DNA molecule to provide target immunity? The mechanism of target immunity is best understood for another transposon, bacteriophage Mu (2, 3), which utilizes two Mu-encoded proteins with different transposition functions: MuA binds specifically to the transposon ends and mediates breaking and joining, while MuB is an ATP-dependent DNA-binding protein that regulates the activity of MuA and is involved in target selection. While a DNA molecule bound by MuB is an efficient transposition target, a DNA molecule that already contains a Mu end is an inefficient transposition target because MuB is cleared from that DNA, leading to inactivation of that target. Clearing of MuB from that target DNA is an example of action at a distance resulting from physical contacts between two DNA sites: MuA protein bound to end-sequences in the target DNA encounters MuB bound elsewhere in that DNA, promoting ATP hydrolysis by MuB and subsequent release of MuB from that DNA (2). Therefore, the role of the transposon end-sequences in an immune target DNA is to increase the effective concentration of MuA, facilitating an encounter with MuB bound elsewhere to that DNA. Indeed, a DNA molecule lacking a Mu end can acquire target immunity by catenation with another DNA molecule containing a Mu end (3). Thus, Mu target immunity works by clearing a DNA of the transposition protein that otherwise promotes insertion when bound to that DNA.

It is not unreasonable to expect that the global features of Tn*7* target immunity are related to Mu. In both systems there are transposition proteins that bind specifically to the transposon ends, while others act at the insertion site in the target DNA, and an ATP cofactor has also been shown to be involved in Tn*7* target immunity in a reconstituted in vitro transposition system (7). Regardless of the underlying molecular mechanisms, we anticipate that Tn*7* target immunity, like Mu, reflects inactivation of the target DNA, resulting from physical contacts between the proteins bound to the transposon sequences that provide target immunity and the proteins bound to the insertion site in that same DNA (32).

How are the Tn*7* sequences that are necessary for target immunity related to sequences in the Tn*7* ends that participate in transposition? A mini-Tn*7* element containing the full complement of TnsB binding sites that are essential for Tn*7* transposition can effectively provide target immunity. We have found that a deletion of one of the four TnsB binding sites from the right end of mini-Tn*7* reduces both the ability to transpose and the ability to provide target immunity, suggesting that the two activities are interrelated. However, while target immunity requires the sequences that participate in Tn*7* transposition, we have found that transposition activity per se is not essential: an immobile mini-Tn*7* element can effectively provide target immunity though it cannot transpose. Thus, only a subset of sequences within the Tn*7* ends that participate in transposition appear to be involved in target immunity.

We believe that Tn*7* target immunity is a useful tool to study features of the chromosome that influence communication between widely separated DNA sites. Our findings support the view that DNA spacing determines the degree of the target immunity effect in a 190-kb region of the *E. coli* chromosome. It will be interesting to use Tn*7* transposition and target immunity to examine at high resolution the contribution of DNA spacing to communication, for example, at 5-kb intervals. It will also be interesting to compare results from the Tn*7* system with those for some other recombination systems which have already been used to evaluate long-distance DNA interactions in vivo: the length dependence of IS*1*-flanked transposons (10), Tn*10* transposition (25), bacteriophage Mu transposition and replication (13), Tn*10* transposase-mediated chromosomal inversions (19), and γδ resolution system-mediated chromosomal deletions (17). A caveat of using a recombination system

to evaluate long-distance DNA communication in vivo is that the experiments can reflect indirect information about chromosome structure; any particular cellular input may evoke a different degree of response in each system. Indeed, this may be a reasonable explanation for why the magnitudes of distance effects are different for the various recombination systems that have already been examined. That is, the penalty for increasing DNA spacing depends on which recombination system is used to evaluate long-distance DNA interactions. Furthermore, it will be important to see if the effectiveness of the Tn*7* long-range DNA interactions is the same at other chromosomal regions.

The *E. coli* chromosome appears to be segregated into about 50 domains of supercoiling (31, 37). Are these topologically independent domains organized at fixed positions in the chromosome? Although the physical forces needed to constrain supercoiling between adjacent domains might impede effective DNA interactions across the domain boundaries, our data provide no evidence for such a static barrier in the *E. coli* chromosome; distributing 50 domains around the *E. coli* chromosome (4,700 kb) would entail boundaries at intervals of about 100 kb, and we have shown here that the long-range DNA interactions necessary for target immunity are effective over at least 190 kb. Another recombination system, examining γδ resolution system-mediated chromosomal deletions (17), also failed to detect any permanent defined boundaries in a 50- to 100-kb region of the *Salmonella typhimurium* chromosome. In contrast, Tn*10* transposase-mediated chromosomal inversions in *S. typhimurium* (19) appear to preferentially select endpoints 40 to 80 kb away, suggesting that the spatial proximity of these preferred target regions is determined by higher-order chromosome structure. In studies using the Tn*7* system, it will be interesting to see if any evidence for static barriers can be found in other regions of the *E. coli* chromosome. Tn*7* target immunity should also be useful as a tool to isolate host mutants which identify components involved in the architecture of the chromosome.

What is the biological significance of target immunity? In this study, we have focused on the effect of target immunity during intermolecular transposition of Tn*7* from one DNA molecule to another DNA molecule. However, a critical regulatory role for target immunity may be to prevent intramolecular transposition of Tn*7* into itself. Intramolecular insertion can result in a disruption of transposon sequences and a break in the DNA backbone containing Tn*7*. It is appealing that a major reason for target immunity is to inhibit insertions into DNA sequences nearest a transposon, avoiding the catastrophic consequences of intramolecular transposition. Substantial regions of the chromosome, and plasmids in particular, would benefit from *cis* interactions that regulate Tn*7* insertion as far away as 190 kb.

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