Negative and positive regulation of \( \text{Tn10/IS10} \)-promoted recombination by IHF: two distinguishable processes inhibit transposition off of multicopy plasmid replicons and activate chromosomal events that favor evolution of new transposons

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\( \text{Tn10} \) is a composite transposon; inverted repeats of insertion sequence IS10 flank a tetracycline-resistance determinant. Previous work has identified several regulatory processes that modulate the interaction between \( \text{Tn10} \) and its host. Among these, host-specified DNA adenine methylation, an IS10-encoded antisense RNA and preferential \textit{cis} action of transposase are particularly important. We now find that the accessory host protein IHF and the sequences that encode the IHF-binding site in IS10 are also important regulators of the \( \text{Tn10} \) transposition reaction in vivo and that these determinants are involved in two distinguishable regulatory processes. First, IHF and the IHF-binding site of IS10, together with other host components (e.g., HU), negatively regulate the normal intermolecular transposition process. Such negative regulation is prominent only for elements present on multicopy plasmid replicons. This multicopy plasmid-specific regulation involves effects both on the transposition reaction per se and on transposase gene expression. Second, specific interaction of IHF with its binding site stimulates transposon-promoted chromosome rearrangements but not transposition of a short \( \text{Tn10} \)-length chromosomal element. However, additional considerations predict that IHF action should favor chromosomal transposition for very long composite elements. On the basis of these and other observations we propose that, for chromosomal events, the major role of IHF is to promote the evolution of new IS10-based composite transposons.

\[ \text{[Key Words: IHF; Tn10; transposon; evolution; plasmid; chromosome rearrangements]} \]

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\( \text{Tn10} \) is a composite transposon; it comprises IS10 insertion sequences that flank tetracycline-resistance genes (Fig. 1; Kleckner 1989). IS10 is an independent transposition module that encodes a single transposase protein. The two ends of IS10 share a nearly perfect terminal inverted repeat, the transposase-binding site but are genetically distinguished as a consequence of base-pair differences within that site and by the presence of a binding site for \( \text{E. coli} \) integration host factor [IHF] adjacent to the outside end-terminal inverted repeat (Fig. 1; Huisman et al. 1989).

\( \text{Tn10} \) and IS10 transpose nonreplicatively. The element is excised from the donor site and inserted into a new target site (Bender and Kleckner 1986). The two chromosome ends left behind by \( \text{Tn10} \) transposon excision are not directly rejoined (Bender et al. 1991). The donor chromosome is presumably either degraded and/or repaired by a recombinational mechanism with information from a sister chromosome (Bender and Kleckner 1986; Bender et al. 1991). The transposition reaction can be subdivided into four successive stages, each dependent on the one before: [1] formation of a synaptic complex between the transposon ends; [2] double-strand cleavage at the two ends of the element; [3] noncovalent capture of a target DNA; and [4] strand transfer of transposon ends to target DNA (Haniford et al. 1991; Benjamin and Kleckner 1992; Chalmers and Kleckner 1994; J. Sakai and N. Kleckner, in prep.).

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Figure 1. Structures of Tn10, IS10, and relevant mini-transposon ends. O + consists of wild-type IS10 base pairs 1–70 (Halling et al. 1982); sequences adjacent to base pair 1 correspond to those adjacent to IS10-Right in hisG9424::Tn10 (Halling and Kleckner 1982); sequences beyond base pair 70 are specific linker base pairs. O-dn is O + with three AT>TA transversions at three consensus base pairs in the IHF-binding site (Huisman et al. 1989); OΔ27 is O + with a deletion from base pairs 28–70; O/123 is the same as OΔ27 except that the deletion extends from base pairs 24–70; O/1 is the same as OΔ27 except that base pairs 24–27 are those found at the corresponding position in the inverted repeat at the inside end of IS10; I27, 12,3, and I19 are the same as O + except that the only transposon sequences present are the first 27, 23, and 19 bp from the inside end of IS10-Right (base pairs 1329–1303, 1307, and 1311, respectively); I/O and I/O-dn are the same as O + and O-dn except that the first 23 bp of the inside end (base pairs 1329–1307) have been substituted for outside-end base pairs 1–23. The outside end of IS10-Left is slightly different in sequence from the outside end of IS10-Right and is thus designated L rather than O.

Tn10 and IS10 not only undergo transposition but also promote chromosome rearrangements. Tn10 promotes both deletion and inversion of adjacent flanking sequences by events in which the two internal ends of the element interact with an adjacent target site in a random collision process (Fig. 2, Kleckner et al. 1979; Benjamin and Kleckner 1992). Information originally located between the inside ends is lost and a segment extending from one inside end to an adjacent target site is inverted, such that a pair of IS10 elements oriented as direct repeats now flank the previously adjacent chromosomal segment. Inversion events promoted in this way by pairs of IS sequences are of particular biological importance because they generate composite transposable elements encoding new genetic determinants [Fig. 2; Wolf 1980; Foster et al. 1981; Raleigh and Kleckner 1984; Navas et al. 1985; Roberts 1986].

Many types of IS elements and their composite transposons appear to be evolutionarily successful, stable components of bacterial genomes [e.g., Lawrence et al. 1989; Biseric and Ochman 1993; Romero and Klaenhammer 1993; Stanley et al. 1993]. The perpetuation of such a relationship requires that effects of transposition deleterious to the host be minimized while sufficient transposition activity is maintained to ensure maintenance of the transposable element. Tn10 and IS10 transposition are known to be subject to regulation or modulation in a number of different ways that can be rationalized on the basis of their advantages for the transposon/host relationship (for review, see Kleckner 1990a,b).

Here, we address the biological roles of the accessory host factor IHF in Tn10/IS10 activity in vivo. An IHF-binding site consensus sequence of moderate strength occurs immediately adjacent to the transposase-binding site at the outside end of IS10 [Fig. 1; Huisman et al. 1989]. Previous work suggested that IHF can affect both transposase expression and the transposition reaction per se but the exact nature of these roles has been unclear [Roberts 1986; Roberts et al. 1987; Morisato and Kleckner 1987; Huisman et al. 1989; D. Morisato, H. Benjamin, and R. Chalmers, unpubl.].

IHF and its relative host component (HU) are small DNA-binding proteins that mediate changes in the structure of DNA. IHF binds tightly to a specific consensus sequence, making a 140° bend in the DNA (Yang and Nash 1989; Nash 1990). HU binds weakly and relatively nonspecifically to DNA but prefers sequences that are naturally bent or flexible (Broyles and Pettijohn 1986; Yang and Nash 1989; Nash 1990). HU binds weakly and relatively nonspecifically to DNA but prefers sequences that are naturally bent or flexible (Broyles and Pettijohn 1986;
To permit analysis of changes in the transposition protein-binding site at the outside end of
been carried out with minitransposons comprising a variety of wild-type and mutant
classical roles, transposase has been provided at varying lev-
ments have all been assayed in IHF + and IHF- strains.
rend-1promoted chromosomal rearrange-
also considered briefly. When
region of the transposase gene promoter
als and methods).
In most experiments transposase was provided by a
which confers sensitivity to sucrose [Gay et
A
the flanking ‘kan’ and ‘ery’ markers [Fig. 3B; Ma-
ments of transposase were examined, and one experiment
compares expression from
with wild-type ‘is10’ making its own transposase [Way
In some experiments higher or lower effective lev-
el of transposase were examined, and one experiment
compares expression from
with wild-type transposasc gene promoter, P-1N.
Transposition and chromosome rearrangements have
both been examined in several different strain back-
grounds. Results are reported here primarily for two
strain backgrounds, NK7419 and NK8087 and appro-
ate isogenic IHF- derivatives; in one set of experiments,
two additional strain backgrounds were examined,
and their IHF derivatives [Materials
Tn10 activity was examined in most detail for con-
structs comprising two IS10-Right outside ends. The
activity of wild-type outside ends (O*) was compared with
the activity of several altered ends compromised with
respect to the IHF-binding site [Fig. 1]. Mutant ends ei-
ther carry an inactivating point mutation in the IHF-
binding site (O-dn) or are deleted for the IHF-binding site
(O27 and O23), and a hybrid end carries a few inside-
end base pairs in place of the IHF-binding site (O/l).
Some experiments examined isogenic constructs com-
prising two inside ends [l27, l23, or l19] or hybrid ends
consisting of the inside end-terminal inverted repeat
plus adjacent end sequences encoding the IHF-binding
site [l/O and the mutant derivative l/O-dn].
In general, for each activity assay, the relative activi-
ties observed for the four genetic situations analyzed,
wild-type end, and any particular mutant end in both
IHF + and IHF- hosts, are presented as a rectangular ar-
ray [Fig. 4A]. The wild-type and doubly defective cases
occupy the upper left and lower right corners of the rect-
angle and the singly defective cases occupy the other two
corners. The ratio of the frequencies observed for five

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**Results**

**Assays for transposition and chromosome rearrangements**

Endogenous intermolecular transposition of Tn10 has
been assayed by a gene activation assay in which transpos-
position results in expression of a suitably positioned
‘kan’ gene lacking transcription and translational start
signals [Bender et al. 1991]. A ‘kan ery’ minitransposon
was used [Fig. 3A]. The identical constructs, together
with adjacent flanking sequences, were examined both
on a multicopy pBR322-based plasmid replicon and in
the chromosome via isogenic single copy λ prophages
[Materials and methods].

Chromosome rearrangements have been monitored by
a newly developed version of the classical assay. A pair of
transposon ends oriented to mimic the inner ends of
Tn10 flank a pair of markers whose loss can be selected
[Fig. 3B]. The transposon ends in such constructs, as well
as the immediately flanking adjacent sequences, are ex-
actly the ‘kan’ and ‘ery’ halves of the mini-transposon used
for transposition assays. Thus, in so far as is possible,
chromosome rearrangements have been assayed with
constructs that are genetically identical to those used to
monitor transposition. The intervening markers are a
sacB gene, which confers sensitivity to sucrose [Gay et
1985] and a λ cI repressor gene, which confers sensi-
tivity to tetracycline via a repressor-sensitive Ptac-tet
reporter fusion present on a separate replicon. The fre-
quency of Tn10-promoted rearrangements is determined
by selection for resistance to sucrose and/or tetracycline.
Among such derivatives, the proportions of deletions
and inversions are determined by scoring for the pre-
ence of the flanking ‘kan’ and ‘ery’ markers [Fig. 3B; Mat-
ergensics and methods].
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Figure 3. Transposon activity assays. (A) Transposition of a mini Tn10-kan eryR transposon from an appropriate donor site to an appropriate new insertion site confers resistance to kanamycin (Bender et al. 1991). The ‘kan segment comprises a nearly complete coding sequence lacking transcriptional and translational start signals. This segment is positioned with the proximal end of the gene located near one end of the transposon and adjacent terminal sequences are devoid of stop codons in-frame to the ‘kan-coding sequence. Insertion of this element into an actively expressed gene in appropriate orientation and reading frame will result in expression of the kan polypeptide. Insertions (1/500–1/1000) of this element into the E. coli chromosome confer kanamycin resistance (J. Mahillon and N. Kleckner, unpubl.). (B) Chromosome rearrangements are promoted by a pair of transposon ends oriented to mimic the innermost ends of a composite transposon, and the frequency of such rearrangements can be measured by determination of the frequency with which the segment between those ends is lost (Kleckner et al. 1979). In the construct shown, the intervening segment confers sensitivity to both sucrose and tetracycline, and rearrangement frequency is determined by selection for resistance to one or both agents (Materials and methods). Deletions and inversion are distinguished from each other by the presence or absence of erythromycin resistance, monitored phenotypically, and/or the ‘kan segment, monitored by colony hybridization.

In each array the total effect of eliminating both IHF and the IHF-binding site is given by the direction and magnitude of the effect shown on the diagonal, that is, the comparison between the fully wild-type and doubly defective cases. The top horizontal and left-most vertical comparisons describe the consequences of eliminating either determinant singly, and the bottom horizontal and right-most vertical comparisons reveals the consequences of eliminating the second determinant when the first determinant has already been eliminated.

Transposition of an outside-end minitransposon off of a multicopy plasmid increases in the absence of both IHF and the IS10 IHF-binding site

At the standard transposase level in NK7419, elimination of both IHF and the IS10 IHF-binding site results in an increase of ~30-fold in the level of Tn10 transposition from a multicopy plasmid (Fig. 4B). The same effect is observed with both O-dn and O/I ends. The occurrence of such an increase was unexpected. It implies that transposition is normally subject to inhibitory effects that are dependent upon the two relevant determinants. We interpret these observations to mean that IHF and its cognate binding site participate in some type of biologically significant negative regulation.

Negative regulation of plasmid transposition involves multiple determinants

Negative regulation of plasmid transposition involves additional complexities. Transposition of outside end elements increases only when IHF activity and the IHF-binding site are both compromised (Fig. 4B). Elimination of IHF function causes only a marginal (approximately twofold) increase in transposition of a wild-type element; and in a wild-type strain, elimination of the IHF-binding site by point mutation or by deletion has essentially no effect. Thus, negative regulation involves one or more factors other than specific binding of IHF to its site. The observed pattern of effects can be explained by the supposition that HU can substitute for IHF in the presence of a wild-type IHF-binding site but not when the IHF-binding site is compromised and that IHF either still mediates negative regulation in the absence of an IHF-binding site (not such an unlikely possibility; see Discussion) or can act indirectly to promote regulation by HU.

Furthermore, a very low transposase level appears to sensitize the transposition reaction to negative regulatory processes (Fig. 5). At transposase levels ranging from...
IHF regulates Tn10/IS10 transposition

(A) Convention for presentation of data. Relative activities of a wild-type outside end [O+] and any given variant outside end in isogenic IHF− and IHF− strains are measured. For each such set of comparisons, five numbers are presented in the diagramed rectangular array. The five values given are the quotients of the frequencies obtained in the five corresponding pairs of situations. The arrow points towards the higher number of the pair. Of particular interest is the number present on the diagonal, which is the ratio of the frequencies obtained in the fully wild-type and doubly defective cases, and reveals the consequences of eliminating both IHF and the IHF-binding site in IS10. Numbers on the horizontal and vertical axes represent the effects of eliminating the two determinants sequentially in the two possible orders. The left-most pair represents the effects of each single defect in the presence of the other determinant, and the right-most pair represents the effects of eliminating the second determinant after the first determinant has already been eliminated. In principle, the number along the diagonal should be the same as the product of each pair of horizontal and vertical numbers. When this is not the case, the differences arise from the rounding off of component numbers in the latter comparisons. The averaged data from two or more experiments represents the averages of the five comparison numbers calculated for the component experiments. In cases where the comparison number fluctuates around the number one, the average is presented as 1. (B) Intermolecular transposition from a multicopy plasmid. [NK7419 and NK8084] that also harbored a Ptac-transposase plasmid pNK3358. The Ptac promoter is probably not fully induced in these experiments, the host strain is lacI-. No external inducing agent [lactose or IPTG] was present at any point. Data are presented for four different experiments and the average of the four. Data for each experiment represent the average of three 2x 10 s, 2x 10 s, 1x 10 −, and 2x 10 s. The background frequencies of kanamycin-resistant derivatives observed for the wild-type transposon in the IHF− strain (the upper left corner of each diagram) for the four experiments were, from left to right, 2x10 −5, 2x10 −5, 1x10 −5, and 2x10 −5. The background frequencies of kanamycin-resistant derivatives observed for the wild-type transposon in the IHF− and IHF− strains in the absence of transposase, i.e., in the presence of an isogenic Tase− plasmid pNK2824 were always <10 −9, well below the lowest levels observed in the presence of transposase. [C] Comparison of I/O and I/O−dn ends in NK7419, NK7814, and DRC325 (and their isogenic IHF− derivatives NK8084, NK7817, and DRC324). In these experiments, transposase was provided in cis by an insertion of the Ptac-transposase fusion into the plasmids carrying the ends [I/O = pNK2820, I/O−dn = pNK2816]. This results in a much higher effective level of transposase because transposase is preferentially cis acting (Morisato et al. 1983; Jain and Kleckner 1993), but the presence of a higher level does not significantly affect the effects observed, at least for outside end elements [Fig. 6]. Frequencies of kanamycin-resistant derivatives observed for the I/O transposon in the IHF− strains were (left to right): 4.6x10 −6, 4.6x10 −6, 1.8x10 −4, 4.3x10 −6, and 5x10 −6; background observed in the absence of transposase [pNK2824] was <10 −9.

0.1 to 30 times the standard level, the observed increases in transposition are similar in each of the two strain backgrounds examined. But at a very low transposase level, elimination of IHF and the IHF-binding site causes an exaggeration in the increase in transposition. At 0.01 times the standard level of transposase, transposition increases ~100- and ~1000-fold, respectively, in the two strain backgrounds.

IS10 inside ends are also subject to negative regulation

IHF-mediated negative regulation of plasmid transposition is also observed with minitransposon constructs comprising inside IS10 ends. For I27, I23, and I19 ends, which naturally lack any IHF-binding site, transposition in an IHF− strain is increased 5- to 20-fold as compared with IHF+ [Table 1]. Moreover, addition of an IHF-bind-

Transposition of outside-end elements off of the bacterial chromosome is affected only marginally by negative regulation

The prominent negative regulation observed on multicopy plasmids is minimal but discernible for transposition from the chromosome. At the standard transposase level in the NK7419 strain background used most extensively for plasmid assays, elimination of IHF and alteration of the IHF-binding site in combination cause chromosomal transposition to increase about twofold [Fig. 6]. This modest increase is real. A similar increase has been
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Table 1. Transposition of minitransposons from a multicopy plasmid in IHF+ and IHF− strains

| End genotype | IHF+ | IHF− | IHF−/IHF+ |
|--------------|------|------|-----------|
| O+           | 1    | 2.8  | 2.8       |
| I27+         | 0.04 | 0.2  | 5         |
| I25+         | 0.016| 0.17 | 11        |
| I19+         | 0.013| 0.09 | 6.9       |

Transposase was provided in cis by a Ptac–transposase fusion present on the same plasmid as the transposon ends. Transposition was assayed in NK7419 and NK8084 exactly as in Figs. 4 and 5. Plasmids were pNK2811 and pNK2817-2819. Data represent the average of three independent experiments, all of which gave very similar results. The absolute frequency of KanR derivatives for the O+ case was $1.8 \times 10^{-4}$; the frequency of KanR derivatives observed with an isogenic plasmid lacking transposase (pNK2824) was $<10^{-9}$. Inside-end activity is inhibited by GATC methylation in these Dam+ strains (Roberts et al. 1985).

Tn10-promoted chromosome rearrangements are specifically activated by IHF-acting at the IHF-binding site

Chromosome rearrangements exhibit yet a third pattern of responses to genetic perturbation of the IHF/end in-

Figure 5. Intermolecular transposition from a multicopy plasmid at varying levels of transposase. Mini-Tn10:kan eryR plasmid constructs were assayed for transposition exactly as in Fig. 4 but at four different levels of transposase. Experiments are presented in two different strain backgrounds. Data for NK7419 and NK8084 (top) represents the average of four independent experiments. Data for NK8087 and NK8088 (bottom) is for one experiment. In this and other assays, these two strain backgrounds reproducibly give qualitatively similar but quantitatively distinguishable results. The standard transposase level was provided in trans from the Ptac–transposase plasmid pNK3358 as in previous figures. An ~30-fold higher level of transposase was achieved by inserting the Ptac–transposase fusion into each of the minitransposon plasmids, which were then assayed in the absence of any additional plasmid. A 10-fold reduction in transposase below the standard level was achieved by providing transposase from a weaker promoter; the corresponding Plac–transposase plasmid pNK3362 is otherwise isogenic to the Ptac–transposase plasmid. The host strains carry a single chromosomal copy of the lacI repressor gene, which provides a small degree of repression to the multicopy plasmid promoters. A reduction of ~100-fold in transposase level was achieved by insertion of a lacIQ gene into the Plac plasmid thus conferring essentially full repression. The relative effective levels of transposase provided in each of these situations have been determined previously (Way 1984) and are also apparent from absolute levels of transposition observed in the fully wild-type [IHF+ O+] situation: NK7419: $2 \times 10^{-4}$, $3 \times 10^{-5}$, $7 \times 10^{-7}$, $2 \times 10^{-9}$; NK8087: $3 \times 10^{-3}$, $5 \times 10^{-4}$, $2 \times 10^{-5}$, $5 \times 10^{-6}$. The background level of kanamycin resistance observed in the absence of transposase was $<10^{-9}$.

observed in the NK8087 strain background at the standard transposase level and in both strain backgrounds at higher and lower transposase levels: in eight additional such experiments, increases varied from zero to fourfold (data not shown).

The chromosomal situation exhibits another interest-

Figure 6. Intermolecular transposition from the chromosome. The same mini-Tn10:kan eryR constructs assayed on plasmids in Figs. 4–6 were assayed in the chromosome on single copy λ prophages [Materials and methods]. Transposase was provided at the standard level, in trans from the Ptac–transposase plasmid pNK3384. Results from two independent experiments involving NK7419 and NK8084, and their average, are shown. The absolute frequencies of kanamycin-resistant derivatives observed for the wild-type transposon in the IHF+ strain were $2 \times 10^{-6}$ and $5 \times 10^{-6}$. The background frequency of kanamycin-resistant derivatives obtained in the absence of transposase was $<10^{-9}$. 

Relative T'ase level

|            | 30 | 1* | 0.1 | 0.01 |
|------------|----|----|-----|------|
| NK7419 O+ and O-dn |
| 1          | 1.2| 2.0| 5   | 3.6 |
| 1.1        | 3.3| 4.4| 3   | 4.1 |
| 1.2        | 6.6| 2.7| 14  | 11  |

NK8087 O+ and O-dn

|            | 1.7| 2.0| 5   | 9.5 |
|------------|----|----|-----|-----|
| 1.2        | 3.3| 4.4| 3   | 4.1 |
| 1.1        | 6.6| 2.7| 14  | 11  |

End Host

Transposition of minitransposons from a multicopy plasmid in IHF+ and IHF− strains

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teraction: They are specifically dependent on both IHF and its binding site.

In the absence of both relevant determinants, deletions and inversions both decrease in frequency. The extent of the decrease observed for the two types of rearrangements taken together ranges from 3- to 13-fold [Fig. 7]. This decrease appears to be the result of the elimination of a simple positive effect of IHF binding to its specific site, irrespective of other factors. Elimination of either determinant singly confers nearly as great a decrease as elimination of both determinants (compare the leftmost horizontal and vertical comparisons with the diagonal comparison).

An additional feature of the data hints, however, at the presence of additional complexities: The effect of eliminating determinants is reproducibly twofold greater for inversions than for deletions [Fig. 7; summary on right]. Random collision between synapsed transposon ends and target DNA is expected to yield deletions and inversions with equal frequency; in this pathway, the two events are mechanistically indistinguishable, differing only in the orientation with which the target DNA happens to interact with the ends. Correspondingly, any genetic alteration that affects this collision pathway is expected to affect both types of events to the same extent. The observation that inversions are affected to a greater extent than deletions is consistent with the existence of an IHF-dependent pathway that leads only to inversions [a channeling pathway; see Discussion].

![Table 7](https://example.com/table7.png)

|        | Del+ Inv | Del Inv | Decrease from double defect |
|--------|----------|---------|-----------------------------|
| O−dn   |          |         |                             |
| x1     | 5 8      | 8 5 7   | 12 7 16                     |
| x2     | 5 8      | 8 5 7   | 5 3 6                       |
| OΔ27   |          |         |                             |
| x1     | 6 4 7    | 4 2 5   |                             |
| x2     | 6 4 7    | 4 2 5   |                             |
| O/I    |          |         |                             |
| x1     | 3 2 3    | 4 2 4   |                             |
| x2     | 3 2 3    | 4 2 4   |                             |

Figure 7. Assays of chromosome rearrangements. Rearrange-
tment tester elements of the type described in Fig. 3 were made carrying O+ and the three types of mutant ends shown, intro-
duced into the bacterial chromosome on single-copy λ pro-
phages and analyzed in the presence of transposase at the stan-
dard level, provided in trans from the Ptae-transposase plasmid
pNK3384 [Materials and methods, Fig. 3B]. X1 and X2 are two
independent experiments in the NK7419 strain background. In
the two experiments, different strains were used that differ with
respect to the orientation of the sacB gene within the tester
construct: X1 and X2 involved constructs bearing the sacB gene
in orientation I and orientation II, respectively [Materials and
methods]. Similar results have been obtained in the NK8087
strain background [data not shown]. The absolute frequencies of
SucR TetR derivatives observed for the construct having O+ ends
in the IHF+ strain for the two experiments were 0.5 × 10−3
[X1] and 1.3 × 10−3 [X2]; the proportions of inversions and dele-
tions in these two cases were 78% and 22% [X1] and 84% and
16% [X2]. The background levels of SucR TetR derivatives ob-
tained for this construct in the presence of a Tase-plasmid,
pNK3386, were 2 × 10−6 [X1] and 7 × 10−7 [X2], two or more
orders of magnitude lower than the lowest levels observed in
the presence of transposase.

Constructs analogous to Tn10 and IS10 respond like O+ constructs to elimination of IHF

The combinations of IS10 ends that occur in nature in-
clude the pair of slightly different outside ends found in
wild-type Tn10 [LxO] and the combination of inside and
outside ends found in IS10-Right [IxO] as well as the
combination of two inside ends [which are identical for
IS10-Right and IS10-Left; Halling et al. 1982]. Thus,
minitransposon constructs corresponding to Tn10 and
IS10 have been analyzed. Both constructs behave simi-
larly to the model outside-end constructs described
above. With respect to intermolecular transposition
from a plasmid, elimination of IHF function confers the
same ~10-fold increase in on the wild-type Tn10 analog
[LxO] as on the O+ [OxO] element analyzed above. With
respect to chromosomal rearrangements, Tn10 [LxO]
and IS10-Right [IxO] constructs exhibit the same ~5- to
10-fold reduction as the (OxO) element.

Also, with respect to chromosome rearrangements,
the Tn10 inside-end analog [IxI] exhibits a small [two-
fold] decrease in an IHF− strain as compared with IHF+
[data not shown]. The existence of a small effect on ends
that do not contain an IHF-binding site supports the
view that IHF can act nonspecifically to promote rear-
grangements.

Transposase expression in the chromosome is not
affected by the absence of IHF

In an earlier analysis, Roberts [1986] examined the ef-
effects of an IHF− mutation on the frequency of rearrange-
ments promoted by an IS10-based transposon composed of a
gal operon carrying a galE− point mutation that is
flanked by IS10-Right and IS10-Left in direct repeat. This
transposon, nadA−::Tn10Gal, arose from a nadA−::Tn10 in-
sertion via a Tn10-promoted inversion event, and it has
exactly the structure of Tn-dcba of Figure 2 with deba
corresponding to gal operon sequences [Raleigh and
Kleckner 1984). The inside ends of TnGal involved in promoting chromosome rearrangements are, genetically, the inside end of IS10-Left and the outside end of IS10-Right.

For n adaA::TnGal, elimination of IHF results in a 5- to 10-fold reduction in the frequency of rearrangements (Table 2), precisely the same effect as that reported above for mini-Tn10 (OxL) despite differences in the strain background, the transposon construct, and the decade in which the experiments were performed. More importantly, exactly the same reduction is observed irrespective of whether transposase was provided from the transposon's own pIN promoter or at a much higher level from a Ptac-transposase fusion present on a multicopy plasmid. Taken together with current data, these observations imply that for IS10 elements in the chromosome, the presence or absence of IHF has little or no effect on the level of transposase gene expression.

More direct evidence to this effect is provided by analysis of transposase-lacZ translational fusions in IHF+ and IHF− hosts (D.E. Roberts, pers. comm.). When such a fusion is present on a multicopy (pUC) plasmid, the level of expression in an IHF+ strain is sixfold higher than in IHF−; in contrast, when the fusion is present in the chromosome on a single-copy λ prophage, elimination of IHF causes an increase of approximately twofold in expression. And sevelfold differences between plasmid and chromosome with respect to the effects of IHF elimination are observed when the fusion construct contains a mutation that improves the IHF-binding site (IHF-up; Huisman et al. 1989) or an I/O hybrid terminus with or without the IHF-up mutation.

Discussion

We interpret the results presented above to mean that IHF and its binding site participate in two different regulatory processes, one negative and one positive, which are directed at two different aspects of Tn10/IS10 biology. Thus, as in many other biological systems, appropriate regulation is achieved by the combined effects of opposing positive and negative tendencies.

Negative regulation specifically inhibits plasmid transposition

Tn10 transposition off of a multicopy plasmid is subject to negative regulation at two levels, neither of which significantly affects Tn10 transposition off of the chromosome.

First, as shown here, IHF and its binding site act directly on the reaction process to inhibit intermolecular transposition: For a mini-Tn10 element on a multicopy plasmid donor replicon, elimination of IHF and its cognate binding site at the outside end of IS10 results in a 10- to 30-fold increase in intermolecular transposition. These same genetic perturbations have relatively little effect on transposition from the chromosome.

Second, previous work has shown that, as a separate effect, IHF inhibits transposase expression for an IS10 element on multicopy plasmids (Huisman et al. 1989; Sussman 1992; Ditto et al. 1994). The data presented here suggest that no such inhibition occurs for an IS10 element located in the bacterial chromosome.

Thus, as for several other IS10 regulatory processes, host factor-mediated regulation is directed at both transposase expression and the reaction process per se.

Previous work has identified several general regulatory features that cause transposition frequencies to decrease as the total number of transposon copies per cell increases. Among these, the most important is probably concentration-dependent inhibition of transposase gene expression by an IS10-encoded antisense RNA, in combination with preferential cis action of transposase, this feature is sufficient to keep the total number of transposition events per genome per generation constant over a wide range of transposon copy numbers (Simons and Kleckner 1983, 1988). Despite these controls, however, transposition of an IS element into a multicopy plasmid replicon poses an immediate risk that a cell lineage will undergo an explosion of transposition events. The host factor-mediated regulation described here presumably provides specific additional barriers against this particularly dangerous possibility.

The fact that negative regulation is exaggerated at very low transposase levels should also mean that sporadic, unscheduled expression of a few transposase molecules from a plasmid-borne IS10 element will essentially never lead to the occurrence of transposition.

Mechanism of negative regulation

IHF-mediated negative regulation in vivo appears to correspond to an in vitro phenomenon known as channeling. The transposition process as observed on a supercoiled plasmid minitransposon OxO substrate does not

Table 2. Frequency of Gal+ chromosome rearrangements promoted Tn(Gal) (x 10-5)

| Experiment | Tase plasmida | Strainsb | IHF+ | IHF | IHF+/IHF |
|------------|--------------|----------|------|-----|---------|
| 1          | none         | 46       | 6.6  | 7.0 |         |
| 2          | none         | 42       | 7.2  | 5.8 |         |
|            | Ptac-taseNcoA | 37       | 12   | 3.1 |         |
| 3.         | Ptac-tase    | 1450     | 250  | 5.8 |         |
|            | none         | 50       | 5.7  | 8.8 |         |
|            | Ptac-taseNcoA | 67       | 11   | 6.1 |         |
|            | Ptac-tase    | 1450     | 360  | 4.0 |         |

The gal genes of TnGal carry a galE mutation that renders cells sensitive to galactose. Cultures were grown in LB from single colonies to saturation, diluted, and plated in parallel on minimal media containing either glucose (to measure total colony forming units) or glucose plus galactose (to measure Gal+ colony forming units; Raleigh and Kleckner 1984).

*aPtac-tase is pNK474; Ptac-taseNcoA is pNK534, a deletion derivative of pNK474 that lacks the central portion of the transposase coding region.

*bNK7381 (cat::TnGal) and an isogenic himA::TN10 hip::cat derivative.
require accessory host factors (R. Chalmers and N. Kleckner, unpubl.). Inclusion of physiologically sensible concentrations of either IHF or HU cause a change in the array of products observed: Transposon excision occurs normally, but strand transfer products are predominantly or exclusively unknotted intratransposon inversion circles [Benjamin and Kleckner 1989, H. Benjamin, R. Chalmers and N. Kleckner, unpubl.]. This bias implies that ends are finding target DNA in a topologically constrained way. Intermolecular transpositions and intratransposon events that arise by random collision of ends with target DNA [e.g., deletions] are suppressed.

The correspondence between the in vivo and in vitro phenomena is supported by previous Southern blotting analysis of products generated in vivo from plasmid-borne mini-Tn10 elements in which the only intratransposon products observed were inversion circles, deletion circles, which arise by a collision mechanism, were absent [Benjamin and Kleckner 1989]. This bias for inversions implies that such substrates are subject to strong channeling effects in vivo.

Furthermore, the O+ plasmid substrate described above responds in the expected way to elimination of IHF or the IHF-binding site. The O+ and O/I mutations both result in a 10-fold decrease in the level of inversion circles as determined by Southern blot hybridization of DNA extracted from saturated cultures, whereas in the same cultures, intermolecular transposition was increased in the case of IHF− as compared with IHF+ as shown above (L. Signon, unpubl.). Fourth, all of the complexities of negative regulation observed in vivo either correspond to or are easily rationalized by features of the channeling reaction observed in vitro [A. Guhathakurta, R. Chalmers, H. Benjamin, and N. Kleckner, in prep.]. Specifically, (1) negative regulation by IHF in vivo occurs on both outside and inside ends [above]; the same is true in vitro. (2) For outside IS10 ends that retain the IHF-binding site, elimination of IHF function alone is not sufficient to fully relieve the negative regulation. This observation can be explained if HU can substitute for IHF for negative regulation. And both IHF and HU are effective for channeling on such ends in vitro. (3) Conversely, for outside IS10 ends in an IHF+ strain background, elimination of the IHF-binding site alone is not sufficient to fully relieve the negative regulation. This observation implies that IHF is capable of mediating negative regulation in the absence of an IHF-binding site. And in vitro, IHF can promote channeling on inside ends even though they lack a specific IHF-binding site. (4) Elimination of both IHF and the IHF-binding site fully relieves the negative regulation despite the presence of HU. This observation suggests that HU is capable of mediating channeling only if a wild-type IHF-binding site is present. This is not unlikely because the IHF-binding site not only provides sequence-specific contacts needed for specific IHF binding but also an intrinsic tendency to bend, which should promote binding of HU as well as of IHF. Furthermore, a mutation that increases the affinity of IHF for this site also increases the effectiveness of HU action at this site (D. Morisato and N. Kleckner, unpubl.), thus, it is not unreasonable to suppose that mutations that decrease the affinity of IHF for this site should decrease the effectiveness of HU.

Positive regulation of chromosome rearrangements

Positive regulation by IHF and its binding site is especially prominent for deletions and inversions promoted by a chromosomal element: The same genetic perturbations that lead to an increase in transposition off of a plasmid lead instead to a 5- to 10-fold decrease in the frequency of chromosome rearrangements.

The mechanism of positive regulation is distinct from that of negative regulation. Activation of chromosome rearrangements appears to involve the specific interaction of IHF at its binding site, as elimination of either determinant singly confers essentially the same effect as elimination of both determinants. The activated rearrangements include both inversions and deletions. Thus, activation occurs for events promoted by the same collision pathway that yields intermolecular transpositions, as deletions and inversions are both generated by a process in which synopsis of transposon ends followed by random collision with target DNA [Benjamin and Kleckner 1992, R. Chalmers and N. Kleckner, in prep.].

Interaction of IHF with its binding site should also promote transposition of long composite transposons

The existence of a difference in the genetic dependencies for chromosome rearrangements and for transposition is particularly interesting because both types of events occur by a random collision pathway. Thus, the only apparent difference between the two types of events is the length of the segment between the synapsed ends, which is a few kilobases in the case of transposition but is the size of the Escherichia coli genome in the case of rearrangements (Fig. 2). These considerations suggest that IHF should also be required for transposition when the transposing segment is very long.

Mechanistically, IHF might be specifically required for formation or stabilization of the Tn10 synaptic complex when the segment between the two transposon ends is very long. It would not be surprising if a longer transposon segment imposed special physical stresses or constraints during or after synaptic complex formation.

It is already known that the transposition reaction can be sensitive to transposon length. The frequency of transposition, even for elements the size of Tn10 or less, in a wild-type strain, decreases exponentially with increasing transposon length, ~40% per kilobase [Morisato et al. 1983]. This transposition length dependence is also observed with IS1-based composite transposons (Chandler et al. 1982). In the case of Tn10, further analysis suggests that length dependence arises prior to transposon excision [Flick 1991]. It would be interesting to determine whether this normal length dependence is increased in an IHF− strain and/or with outside ends that lack an IHF-binding site.


Transposition from the chromosome

Chromosomal transposition events are subject to negative regulation, presumably at least in part via channeling. Transposition from the chromosome increases about twofold in the absence of both IHF and a functional binding site. Transposition from the chromosome is also subject to positive regulation: If either IHF or the IHF-binding site is eliminated singly, transposition from the chromosome is markedly decreased.

The level of transposition observed in these singly defective cases is very low. There are two possible explanations for this result. First, the chromosome may be the subject of both strong positive and strong negative regulation, which are essentially independent of one another. Elimination of positive regulation in the singly defective cases reveals the existence of the strong negative regulation, and when both are eliminated, the situation is not very different from when both are present. Second, it is possible that in the wild-type situation, where positive regulation is present, negative regulation is negligible, and that strong negative regulation occurs only in the absence of positive activation.

Some fraction of chromosome rearrangements may be channeled into an inversion-only pathway

Elimination of both IHF and a functional IHF-binding site causes a twofold greater decrease in chromosomal inversions than in chromosomal deletions. In the fully wild-type situation, the ratio of inversions to deletions is \( \sim 4:1 \) (3:1 and 5:1 in X1 and X3, respectively), in the absence of both IHF and its binding site, the ratio of inversions to deletions is \( \sim 2:1 \) (average of all data in Fig. 7; range is 1.3:1 to 2.5:1). Because inversions and deletions that occur by the collision pathway should be mechanistically indistinguishable, differing only in the orientation with which the target DNA and the transposon ends happen to interact, the differential behavior of the two types of rearrangements suggests the existence of some other variable.

One simple possibility would be that in a fully wild-type situation, some inversion events arise by the inversion-specific channeling pathway. The quantitative effects of eliminating both IHF and its binding site are consistent with the possibility that about one-third of all chromosome rearrangements are normally channeled. In the fully wild-type case, 20% of rearrangements are deletions and 80% are inversions (average of X1 and X2; legend to Fig. 7). If all deletions arise by collision and if the ratio of inversions to deletions via the collision pathway [given by the ratio in the absence of IHF and its binding site] is 2:1, then in the wild-type case, 25% of events are collision deletions, 50% are collision inversions, and the remaining 25% of events are inversions that arrive by channeling. This estimate predicts that the ratio of inversions to deletions in the presence and in the absence of channeling should be 2.35:1 (not shown), the observed ratio is 2.3:1.

If this scenario were exactly true, and if the rules for channeling observed on plasmids apply directly to chromosomal events as well, single mutant defects [in IHF or the IHF site] should differentially affect deletions (which arise only by collision) as compared with inversions [which arise by both collision and channeling]. This simple expectation is not borne out.

IHF should specifically promote the formation of new composite transposons

Taken together, the observations presented here suggest that the interaction of IHF with its binding site should play an important role in both the formation and the transposition of new composite transposons. A new transposon can arise in either of two ways, both of which should be stimulated by IHF.

In one process, an individual IS10 element may arise at one or more new locations such that a gene of interest is flanked by two new or one new and one old element. In the most general case, the resulting newly formed transposon encoding the gene of interest will be very long, because both the pre-existing element and any new IS10 insertions will be randomly disposed with respect to that gene (e.g., Wolf 1980). Thus, if IHF generally promotes transposition of long composite elements, it will be particularly important for subsequent transposition of newly formed composite elements. The IHF dependence will eventually be lost as the newly formed transposon evolves. Because transposition is more efficient for shorter elements, newly formed elements will tend to become progressively shorter as they undergo successive rounds of transposition.

In a second process, an existing pair of IS10 elements undergoes a chromosome rearrangement that places new sequences between the elements, as in Tn10-promoted inversions [Fig. 2]. IHF should promote the formation of such rearrangements both by acting as a positive factor for inversions that arise by collision and, perhaps more especially, by specifically channeling some collision events into the topologically constrained pathway that yields only inversions.

Why is a multicopy plasmid different from the chromosome

The only feature of IHF-mediated regulation for which a possible explanation is not immediately available is that plasmid substrates are differentially sensitive to negative regulation of both transposase expression and transposition as compared with chromosomal substrates. An obvious possibility is that the steady-state level of negative supercoiling in plasmids might be higher than for the chromosome, because supercoiling would presumably promote a topologically constrained reaction pathway. In vivo estimates of effective supercoiling levels suggest, however, that plasmid and chromosomal substrates are similar in this regard [Sinden et al. 1980; Bliska and Cozzarelli 1987]. Local supercoiling effects [Liu and Wang 1987] also seem unlikely because different types of con-
structs were used in current and previous [Benjamin and Kleckner 1989] studies.

A second possibility would be that plasmids and chromosomes have different arrays of chromatin structure proteins, perhaps as a consequence of differences in plasmid and chromosomal replication. A third possibility would be that the length of the nontransposon sequences in the substrate molecule affects the nature of the reaction subsequent to synaptic complex formation (e.g., Wang and Harshay 1994).

Materials and methods

Bacterial strains

Strains used for assaying transposition off of plasmids

NK7419, trp his arg met supE44 tonA tss mtlZ yfi7 lacΔ1; NK8084, NK7419 himA::TnlOΔ82 Δhim::cat; NK9087, galK2 thyA strR; NK3086, NK8087 himA::TnlOΔ82 Δhim::cat; himA::TnlOΔ82 and Δhim::cat as described by Miller (1984) and Flamm and Weisberg (1985), respectively, NK7814, W3110 strR lacΔ1; NK7817, NK7814 himA::TnlOΔ82 hipΔ; DRC325, recA56 lacZU169 galK2 rpsL200; DRC324, DRC325 himA ΔSmal Δhim::cat.

Strains used for experiments involving transposons carried as λ prophages

For all experiments that assay chromosome rearrangements and for assays of chromosomal transposition in which transposase was provided in trans, lysogens of the desired λ phages were obtained in NK7419 and/or NK8087 by infection of each phage and selection for a phage-encoded erythromycin resistance marker (see below). After purification, single lysogens were identified by testing (Mouset and Thomas 1969); then an isogenic IHF (hip::cat) version of each desired single lysogen was constructed by PI transduction. The himA::TnlOΔ82 marker was not introduced because it was incompatible with the selection for chromosome rearrangements. All available evidence suggests that elimination of a single IHF subunit is sufficient to eliminate all IHF activity (Werner et al. 1994; Zulianello et al. 1994).

In experiments involving multicopy plasmids, plasmids were introduced in monomeric form into the desired strain by transformation. Fresh transformants were generated for each experiment as the first steps in the procedure (see below). Transposon-bearing plasmids and transposase-bearing plasmids were introduced and maintained by selection for erythromycin and ampicillin resistance, respectively. For experiments involving both types of plasmids, the transposase plasmid was introduced first by selection for ampicillin resistance and the transposon-bearing plasmid was introduced subsequently by selection for erythromycin resistance in the presence of ampicillin.

Plasmids

Multicopy plasmids carrying mini-Tn10:‘kan eryR transposons and a Ptac–transposase gene are derivatives of pBR333 (Foster et al. 1981), a medium copy derivative of pBR322 that retains ampr: pNK2811(O1′), pNK2823(O-Dn), pNK3348(O277), pNK3350(O2423), pNK2812(O1′), pNK2820(I/O), pNK2816(I/O-Dn), pNK2819(l27), pNK2818(l23), and pNK2817(l19). These plasmids were constructed in a single step by ligation of four pieces of DNA: (1) An EcoRI–HindIII backbone fragment from precursor plasmid pLO88, which carries the origin and amp gene of pBR333, the Ptac–transposase gene and a set of transcription terminators derived from pNK1391, the latter segments are embedded within a segment encoding the hisOPGD region of Salmonella typhimurium. [2] An EcoRI–SalI fragment carrying the desired type of IS10 end; [3] A Xhol–BamH1 fragment from pNK3505 carrying the ‘kan and eryR genes; and [4] a BclI–HindIII fragment carrying the desired type of IS10 end. The ends on fragments 2 and 4 may be the same or different. In these constructs, the transposase gene was transcribed from a Ptac promoter located 1.5 kb from and directing transcription away from the nearest transposon end. pNK3505 was constructed as follows. A BamH1–SalI fragment containing the ‘kan gene from pKM109-9 (Reiss et al. 1984) was cloned into pGCI [Myers et al. 1985] to yield pNK3504. Plasmid pG14010 carrying the erythromycin resistance gene [Josson et al. 1989] was digested with SalI and SacI, its overhanging ends filled in, and the resulting fragment was cloned into a filled-in SalI site in pNK3504 to yield pNK3505.

Isogenic transposase-defective derivatives of the above plasmids were constructed in analogous four-piece constructions differing from the above only in that the transposase gene on fragment 1 had been inactivated by digestion with AccI, filling in the overhanging ends, and inserting a SalI 8-mer linker. The corresponding Tacs (plasmids ptNK2824 (O1′), pNK3344(O-Dn), pNK3346(O1′), pNK3354(O23), and pNK3352(O27)).

Plasmids that encode only a transposase gene and no transposon are as follows: The Ptac–transposase plasmid pNK3358 was obtained by the cloning of the BamH1–HindIII fragment of pDHI0 [Hamford et al. 1990] into pACYC177. The Plac–transposase plasmid pNK3362 was obtained by cloning the EcoRI fragment of pNK997 [Shen et al. 1987] into pACYC177. A derivative of pNK3362 containing a lacP gene, pNK3364, was obtained by inserting an EcoRI fragment of pNL67 (obtained from J. Wang, Harvard University, Cambridge, MA) at the EcoRI site of pNK3362.

Assays of chromosome rearrangements involved plasmids that carried both a transposase gene and a Pn-tetR fusion (Roberts et al. 1980). An Avel (filled-in)–HindIII fragment of pTR264 carrying the Pn-tet fusion was cloned into each of the transposase plasmids described above following digestion with HindIII and Smal. pNK3358 (Plac) yielded pNK3354; pNK3362 (Plac) yielded pNK3368; pNK3364 yielded pNK3370. pNK3386 is isogenic to pNK3384 except that it lacks a functional transposase gene; it was constructed by the replacement of the BamH–HindIII fragment of pNK3358 with the corresponding fragment carrying the NcolI allele from pDHI12 [Hamford et al. 1989] after insertion of the Pn–tet fusion fragment. pNK3390 and pNK3392 are isogenic to pNK3368 except that they lack a functional transposase gene; these plasmids were constructed by the replacement of the EcoRI–HindIII fragments of pNK3362 and pNK3370, respectively, with a fragment carrying a Plac–transposase NcolI allele from pNK3362.

Phages

For assays of transposition from the chromosome, transposon constructs were transferred by recombination in vivo from the plasmids described above to a lysogenization-proficient λ phage, λA371 = λ hisGD imm434 ind−.

For assays of chromosome rearrangements, the assay tester construct was first constructed on a plasmid and then transferred to λA371 by recombination in vivo. The tester construct for any given type of ends was obtained in three steps. (1) Four fragments were ligated together: A SalI–PvuII backbone derived from pNK2242 (a derivative of pBR333), an EcoRI–PvuII fragment from the appropriate plasmid of the pNK2811 series (see above) containing one end plus the eryR gene, a HindIII–SalI
fragment also from the appropriate plasmid of the pNK2811 series carrying one end plus the 'kan' gene, and a EcoRI–HindIII ended fragment carrying a PlacUV5–cl fusion gene; the latter fragment was derived by partial digestion of a plasmid similar to pEA306 [Amann et al. 1983]. (2) A Ncol linker was then inserted at the EcoRi site located between the PlacUV5–cl fragment and the adjacent eryR terminus segment, and (3) a Ncol fragment carrying the sacB gene from pBlp (Slater and Maurer 1993) and subcloned into pBluescript. For each construct, plasmids carrying the sacB fragment in both possible orientations were kept. In orientation I, the sacB gene is transcribed in the same direction as the PlacUV5–cl gene; orientation II is the opposite. For orientation I, plasmids are pNK3409 [O+], pNK3411 [OxL], pNK3413 [O–dn], pNK3414 [O+ll], pNK3416 [OxL], pNK3419 [I27], and pNK3418 [O.I27]. For orientation II, the plasmids are pNK3423 [O+], pNK3425 [OxL], pNK3427 [O–dn], pNK3429 [O+ll], pNK3430 [OxL], pNK3432 [I27], and pNK3433 [O.I27]. For orientation I, the corresponding phages are λ1395 [O+], λ1397 [OxL], λ1399 [O–dn], λ1401 [O+ll], λ1405 [O.I27], and λ1407 [I27]. For orientation II, the corresponding phages are λ1409 [O+], λ1411 [OxL], λ1413 [O–dn], λ1415 [O+ll], λ1417 [OxL], λ1419 [I27], and λ1421 [I27].

Transposition and chromosome rearrangement assays

Transposition by 'kan' elements was assayed as follows. For each strain to be tested, three or five single colonies representing primary transformants containing the plasmids of interest were picked from an LB plate containing erythromycin and ampicillin, each colony was used to inoculate 1 ml of LB broth containing ampicillin. Cultures were grown 12 to 14 hr with aeration and then mixed on LB ampicillin plates in the presence and absence of kanamycin. Results presented for each strain in any single experiment are the average of the three to five independent clones assayed.

Chromosome rearrangements were assayed analogously except that the final cultures were streaked on LB ampicillin plates in the presence or absence of tetracycline (for lysogens in NK7419 and their isogenic IHF derivatives) or on LB ampicillin plates in the presence or absence of sucrose (for lysogens in NK8087 and their isogenic IHF derivatives). The desired Tet^R Suc^R derivatives were identified in the former case by replica plating of tetracycline-resistant colonies onto sucrose-containing plates and in the latter case by replica plating of sucrose-resistant colonies onto tetracycline-containing plates. The protocol was varied for NK8087 because this strain exhibited a high background of irrelevant tetracycline resistance. The frequency of Tet^R Suc^R and Suc^R Tet^R colonies was negligible (<6%) in all experiments reported here except in strains lacking transposase in which case only background events occur. In all cases, only Suc^R Tet^R colonies were considered in the frequencies reported.

The presence of the eryR marker was assayed by replica plating all colonies onto LB plates containing erythromycin. This approach is subject to two uncertainties: Very short adjacent deletions that do not extend into the eryR marker would have been scored as inversions and, conversely, inversions to a target site within the eryR marker would have been scored as deletions. Previous analysis of Tn10-promoted rearrangements suggests that such events should constitute a small proportion of the total, however [Kleckner et al. 1975; Raleigh and Kleckner 1984, Shen et al. 1987]. The presence of the 'kan' marker was scored by colony hybridization [Bender et al. 1991].

Methods and media

All experiments were carried out using standard microbiological procedures (Miller 1972); previous publications from the Kleckner laboratory. Antibiotics were used at the following concentrations: 100 µg/ml of ampicillin; 50 µg/ml of kanamycin, 200 µg/ml of erythromycin, 10 µg/ml of tetracycline; and 5% sucrose.
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