Fitness Effects of a Deletion Mutation Increasing Transcription of the 6-Phosphogluconate Dehydrogenase Gene in \textit{Escherichia coli}\textsuperscript{1}

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Directed evolution in microbial organisms provides an experimental approach to molecular evolution in which selective forces can be controlled and favorable mutations analyzed at the molecular level. Here we present an analysis of a mutation selected in \textit{Escherichia coli} in response to growth in a chemostat in which the limiting nutrient was gluconate. The selectively favored mutation, designated \textit{gnd}\textsuperscript{*} (862), occurred in the gene \textit{gnd} coding for 6-phosphogluconate dehydrogenase, used in gluconate metabolism. Although the allele is strongly favored in chemostats in which the limiting nutrient is gluconate, the selective effects of \textit{gnd}\textsuperscript{*} (862) are highly dependent on growth conditions. In chemostats in which growth is limited by a mixture of gluconate and either ribose, glucose, or succinate, the \textit{gnd}\textsuperscript{*} (862) allele is favored, disfavored, or neutral according to the relative concentrations of the substrates. The \textit{gnd}\textsuperscript{*} (862) allele results from a deletion of 385 nucleotide pairs in the region 5' to the promoter of \textit{gnd}, and one endpoint of the deletion is contiguous with the terminus of an IS5 insertion sequence located near \textit{gnd} in \textit{E. coli} K12. The \textit{gnd}\textsuperscript{*} (862) allele shows a marked increase in transcription that accounts for most or all of the increased enzyme activity.

\textbf{Introduction}

A powerful experimental approach for studying molecular evolution is that of directed evolution, in which organisms, typically bacteria, are placed in environmental conditions in which there is strong selection for some novel genetic capability (reviewed in Mortlock 1982; Hall 1983; Hartl and Dykhuizen 1984; Hartl 1986). In the directed-evolution experiments described in the present paper, we cultured, in chemostats in which the limiting nutrient was gluconate, strains of \textit{E. coli} in a genetic background containing a mutation (\textit{edd}) that prevents direct shunting of gluconate through glycolysis. Under these conditions, spontaneous mutations better able to utilize gluconate are strongly favored.

Normally in \textit{E. coli}, phosphorylated gluconate is routed either through the Entner-Doudoroff pathway by phosphogluconate dehydratase, coded by the \textit{edd} gene, or through the pentose shunt by 6-phosphogluconate dehydrogenase (6PGD), coded by the \textit{gnd} gene (Bachmann 1983; Ingraham et al. 1983, pp. 136ff.). Strains of \textit{E. coli} that have a deletion in \textit{edd} divide very slowly in gluconate medium and are strongly favored.

1. Key words: directed evolution, chemostats, \textit{Escherichia coli}, IS5, 6-phosphogluconate dehydrogenase.

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disfavored in chemostats in which gluconate is the limiting nutrient (Dykhuizen et al. 1984). New mutations with higher growth rates quickly take over the chemostat cultures. In one such mutation, the higher growth rate was mainly attributable to a mutation in the \textit{gnd} gene, designated the \textit{gnd}^+(862) allele, which causes a threefold increase in enzymatic activity of 6PGD (Miller et al. 1984). The \textit{gnd}^+(862) allele has an \textasciitilde400-bp deletion 5' to the coding sequence, and subcloning experiments indicate identity in the coding regions of both \textit{gnd}^+(862) and the \textit{gnd}^+(811) allele from which it arose.

In the present paper we provide a detailed molecular characterization of the evolved \textit{gnd}^+(862) allele and demonstrate its unusual fitness characteristics in chemostats. The increased activity of \textit{gnd}' (862) is associated with an increase in transcription, which initiates at the same nucleotide site as in the wild-type allele in \textit{E. coli} K12. At the DNA level, the structural rearrangement in \textit{gnd}^+(862) brings the insertion element IS5 close to the \textit{gnd} promoter. The selective effects of the \textit{gnd}^+(862) allele are unusual in that, in chemostat experiments with competition for gluconate and an alternative carbon source, the allele is favored in some environments, disfavored in others, and neutral in still others.

Material and Methods

Strains and Plasmids

Strains and plasmids used are listed in table 1. All strains have a K12 genetic background. The allele \textit{gnd}^+(811) was derived from the natural isolate RM72B (Milkman 1973) by repeated transductions into the K12 background (Hartl and Dykhuizen 1981; Miller et al. 1984) and was confirmed by electrophoretic mobility of 6PGD. DD812 is an \textit{fhuA} derivative of DD811 containing the identical \textit{gnd}^+(811) allele. All DD strains in table 1 carry \textit{rpsL}. The delta symbol (\textasciitilde) denotes a deletion. Strain RW181 was the host for the plasmids.

The \textit{gnd}^+(862) allele arose in strain DD812 inoculated into gluconate chemostats. DD812 (and the isogenic DD811) contain a copy of IS5 located upstream from \textit{gnd} (Miller et al. 1984). The presence of IS5 at this location is characteristic of most \textit{Escherichia coli} K12 strains, but it is not present in other isolates, including RM72B (Sawyer et al. 1987; Barcak and Wolf 1988). The \textit{gnd}^+(811) allele in strains DD811 and DD812 previously had been denoted \textit{gnd}^+(RM72B) (Hartl and Dykhuizen 1981; Miller et al. 1984) on the basis of the electrophoretic mobility of the 6PGD enzyme. The \textit{gnd}^+(811) allele is actually a recombinant gene combining the upstream regulatory regions of the RM72B and K12 \textit{gnd} alleles (Barcak and Wolf 1988).

The \textit{gnd}^+(811) and \textit{gnd}^+(862) alleles were cloned in pBR322 as fragments extending from the \textit{EcoRI} site in IS5 to the \textit{EcoRI} site located 3' to the \textit{gnd} gene (Miller et al. 1984). These plasmids are pLX5, which contains \textit{gnd}^+(811), and pLX7, which contains \textit{gnd}^+(862). The homologous fragment from a strain containing the K12 gene \textit{gnd}^+ was similarly cloned, and the plasmid was designated pLX3. The restriction map in figure 1 shows the relationship of IS5 to both the coding region of \textit{gnd} and the region shown to be deleted in the evolved gene. The map extends from the \textit{BglII} site in IS5 through the first \textit{KpnI} site in \textit{gnd} and shows the region sequenced from plasmids pLX3, pLX5, and pLX7.

RNA Analysis

RNA preparations were obtained by growing cells to late log phase (150 Klett units) in Luria broth and isolating RNA by phenol extractions at 60 C (Miller 1972,
Table 1

<table>
<thead>
<tr>
<th>Strain or Plasmid</th>
<th>Relevant Genotype</th>
<th>Source and Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>DD725</td>
<td>$\Delta$(eda-edd-zwf) gnd$^\ast$(K12)</td>
<td>Hartl and Dykhuisen 1981</td>
</tr>
<tr>
<td>DD811</td>
<td>$\Delta$(eda-edd-zwf) gnd$^\ast$(811)</td>
<td>Hartl and Dykhuisen 1981</td>
</tr>
<tr>
<td>DD812</td>
<td>$fhuA\Delta$(eda-edd-zwf) gnd$^\ast$(811)</td>
<td>Miller et al. 1984</td>
</tr>
<tr>
<td>D862</td>
<td>$fhuA\Delta$(eda-zwf) gnd$^\ast$(862)</td>
<td>Miller et al. 1984</td>
</tr>
<tr>
<td>DD1660</td>
<td>gnd$^\ast$(811)</td>
<td>Transductant from DD811, eda$^\ast$ edd$^\ast$ zwf$^\ast$ from RM77C</td>
</tr>
<tr>
<td>DD1704</td>
<td>$fhuA$ gnd$^\ast$(862)</td>
<td>Coisogenic with DD1660</td>
</tr>
<tr>
<td>RW181</td>
<td>$\Delta$(edd) $\Delta$(gnd)</td>
<td>Nasoff and Wolf 1980</td>
</tr>
<tr>
<td>WB351</td>
<td>$\Delta$(his-gnd) Sm'/F$:;Tn3</td>
<td>Barnes and Tuley 1983</td>
</tr>
<tr>
<td>pLX2</td>
<td>pBR322 containing gnd$^\ast$(K12)-bearing EcoRI fragment from DD725</td>
<td>Miller et al. 1984</td>
</tr>
<tr>
<td>pLX3</td>
<td>Like pLX2 but opposite orientation</td>
<td>Miller et al. 1984</td>
</tr>
<tr>
<td>pLX4</td>
<td>pBR322 containing gnd$^\ast$(811)-bearing EcoRI fragment from DD811</td>
<td>Miller et al. 1984</td>
</tr>
<tr>
<td>pLX5</td>
<td>Like pLX4 but opposite orientation</td>
<td>Miller et al. 1984</td>
</tr>
<tr>
<td>pLX6</td>
<td>pBR322 containing gnd$^\ast$(862)-bearing EcoRI fragment from DD862</td>
<td>Miller et al. 1984</td>
</tr>
<tr>
<td>pLX7</td>
<td>Like pLX6 but opposite orientation</td>
<td>Miller et al. 1984</td>
</tr>
<tr>
<td>pLX5-2</td>
<td>BamHI-BglII deletion derivative of pLX5</td>
<td></td>
</tr>
<tr>
<td>pLX7-2</td>
<td>BamHI-BglII deletion derivative of pLX7</td>
<td></td>
</tr>
</tbody>
</table>

pp. 328–330). Replicate samples were obtained for each strain. For RNA dot blots, twofold serial dilutions starting with 1.0 µg were applied to nitrocellulose by using a Schleicher and Schuell microsample filtration manifold apparatus. The nitrocellulose was dried and hybridized (Hartl et al. 1983) with a nick-translated $^{32}$P-labeled probe made from the BgIII-to-EcoRI-gnd-containing fragment of pLX5.

For primer extension, a 20-base oligonucleotide corresponding to bases 55–74 of the translated region of the mRNA (fig. 4, nucleotides 693–712) was end labeled

![Restriction map of gnd$^\ast$(811) allele in pLX5](image)

**FIG. 1.—** Restriction map of gnd$^\ast$(811) allele in pLX5. The black bar indicates the 385-bp deletion in the gnd$^\ast$(862) allele in pLX7. The restriction map of the gnd$^\ast$(K12) allele in pLX3 is identical to gnd$^\ast$(811) except that it lacks the PsIL site. The stippling indicates the transcribed region; the wide bar indicates the coding region; and the eight vertical lines indicate the positions of nucleotide differences between gnd$^\ast$(811) and gnd$^\ast$(K12).
using [$\gamma^{32}$P]ATP and T4 polynucleotide kinase, and then 13.2 ng of this oligonucleotide together with 100 µg of bacterial RNA were ethanol precipitated and resuspended in hybridization buffer (Berk and Sharp 1978). The solution was heated to 70 C for 10 min and incubated at 43 C for 3 h. After ethanol precipitation overnight at -20 C, the annealed RNA was resuspended in 19 µl of reverse transcriptase buffer containing deoxynucleotides (Wu et al. 1987) and incubated for 30 min at 42 C with 10 units of AMV reverse transcriptase (Pharmacia). The reaction mixture was ethanol precipitated and resuspended in 10 µl of sequence loading mix. After being heated at 100 C for 5 min and cooled quickly, 3 µl was loaded onto a 6% polyacrylamide sequencing gel.

DNA Sequence Analysis

DNA sequencing was carried out using both the chemical sequencing method (Maxam and Gilbert 1980) and the dideoxy chain-termination method (Sanger et al. 1977). Sanger sequencing was carried out with fragments subcloned into phage M13 vectors (Messing 1983) and utilizing either the universal primer or appropriate synthetic oligonucleotide primers (Strauss et al. 1986). Phage containing sequences derived from K12 strains were grown in JM103 hosts, and those containing gnd sequences from other strains (pLX5 and pLX7) were grown in WB351 hosts (Barnes and Tuley 1983), since the gnd region in WB351 is deleted.

Chemostat Competitions

Chemostat competition experiments were carried out as described elsewhere (Dykhuizen and Hartl 1980) using a generation time of 2.0–2.1 h and a total nutrient concentration (gluconate + ribose or other alternative carbon source) of 0.1 g/liter.

Results

Increased Transcription of gnd+(862) Allele

Previous subcloning experiments had demonstrated that the coding regions in the gnd+(811) and gnd+(862) alleles were identical, and therefore coding differences could not explain the threefold difference in enzymatic activity (Miller et al. 1984). In those experiments, all cloned sequences 5' to the AccI site at position 570 in figure 1 were deleted by digesting the plasmids to completion with AccI, followed by ligation. The ligation joined the AccI site at position 570 with an AccI site located 3.4 kb upstream in the vector (Miller et al. 1984). Strains containing these deletion derivatives had indistinguishable levels of 6PGD expression (table 2), indicating that the region 5' to the AccI site is probably responsible for differences in levels of expression. To further define the sequences responsible for the differences in gnd expression, the BamHI-BglII fragment in pLX5 and pLX7 was deleted, thus eliminating most of the IS5 coding sequences. The differences in expression remained (table 2). Thus, the DNA sequences responsible for the difference in expression are localized to the region between the BglII and AccI sites.

The levels of gnd messenger RNA differ in the gnd+(811) and gnd+(862) alleles, as indicated by the RNA dot blots in figure 2. Relative to the gnd+(811) allele, the evolved gnd+(862) allele has a marked increase in the steady-state level of gnd messenger RNA. We conclude that the increased expression of 6PGD observed in the gnd+(862) allele is due in large part, if not exclusively, to increased transcription. As demonstrated below, the nucleotide sequence of the 6PGD messenger RNA from the gnd+(862) allele is identical to that from gnd+(811).
Table 2
Activity of 6-Phosphogluconate Dehydrogenase

<table>
<thead>
<tr>
<th>SOURCE OF gnd ALLELE</th>
<th>DD811 (pLX5)</th>
<th>DD862 (pLX7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Original plasmid</td>
<td>5.42 ± 0.41</td>
<td>17.03 ± 0.94</td>
</tr>
<tr>
<td>AccI-AccI deletion</td>
<td>4.93 ± 0.27</td>
<td>5.62 ± 0.51</td>
</tr>
<tr>
<td>BarnHI-BglII deletion</td>
<td>5.57 ± 0.54</td>
<td>19.41 ± 1.05</td>
</tr>
</tbody>
</table>

* Constructed by ligation of AccI site at position 570 in fig. 1 with AccI site 3.4 kb upstream in the vector. Relative values from Miller et al. (1984, table 4), normalized to activities in original plasmids.

b Constructed by removal of the small BamHI-BglII fragment, intramolecular ligation of the resulting molecules, and transformation of strain RW181.

One model for the increased gnd messenger RNA in the evolved strain is that the deletion may allow additional transcript(s) from different or altered initiation sites to occur. Two lines of evidence argue against this possibility.

First, RNA protection experiments showed neither detectable heterogeneity in messenger RNA length in the evolved strain nor difference in the length of messenger RNA. The protection experiments were carried out using RNA isolated from strains bearing the gnd plasmids pLX3, pLX5, and pLX7. The DNA fragment hybridizing with RNA and thereby protected from digestion by single-stranded nuclease was indistinguishable in size in all strains (data not shown).

Second, primer extension experiments showed that transcription initiates at identical nucleotide sites in the three alleles of gnd (fig. 3). The common initiation site is identical to that found by Nasoff et al. (1984) for the wild-type allele in Escherichia coli K12.

DNA Sequence of gnd+ (862) and gnd+ (811) Alleles

To define the molecular basis of the regulatory mutation in gnd+ (862), the region between the BglII site in IS5 and the KpnI site in gnd was sequenced in all three plasmids. Plasmid pLX3 [gnd+ (K12)] contains 1,013 nucleotides in this region (fig. 4). The 158-bp region sequenced in IS5 is identical with the published sequence (Kroger and Hobom 1982), and the IS5 element is oriented in the genome with the long open reading frame transcribed in the same counterclockwise direction as gnd. The 388-bp region between IS5 and gnd is very A/T rich, with two runs of five or more A's and three runs of five or more T's. The conventional regulatory region beginning with the −35 site is 92 bp in length, and the sequenced portion of the translated region is 375 bp. The sequence of gnd+ (K12) is identical with that reported by Nasoff et al. (1984) in the regulatory and translated regions. However, part of our sequence from E. coli K12 differs substantially from that reported by Nasoff et al. (1984).

The sequence of gnd+ (811) in plasmid pLX5 is identical with that of gnd+ (K12) except for eight single-nucleotide differences in the region 902–1004, which are shown as vertical bars in the restriction map in figure 1. The differences are also indicated by the capital letters above the sequence of gnd+ (K12) in figure 4. All eight of the differences between the two alleles are silent substitutions.

The sequence of the evolved allele gnd+ (862) in plasmid pLX7 is identical to that of the original allele gnd+ (811) except for the deletion of 385 nucleotides. The
deletion extends from the nucleotide immediately adjacent to IS5 through a position in the −35 region four nucleotides 5′ to the *Accl* site (figs. 1, 4).

Selective Advantage of *gnd*<sup>+</sup>(862) Allele

To investigate conditions in which the *gnd*<sup>+</sup>(862) allele has a selective advantage, chemostat competition experiments were carried out between isogenic strains DD1660 [*gnd*<sup>+</sup>(862)] and DD1704 [*gnd*<sup>+</sup>(811)]. Initial competition experiments with gluconate as the limiting nutrient indicated that *gnd*<sup>+</sup>(862) had a selective advantage of ~3.8%/h. As expected, in chemostats limited for ribose, *gnd*<sup>+</sup>(862) and *gnd*<sup>+</sup>(811) were indistinguishable. Ribose was chosen as an alternative carbon source because
ribose-5-phosphate is the principal product of the pentose shunt in which 6PGD participates.

To determine the selective advantage of gnd\textsuperscript{+} (862) as a function of gluconate concentration, we carried out chemostat competitions by using a mixture of gluconate and ribose. Figure 5 shows the results, which were quite unexpected in that the gnd\textsuperscript{+} (862) allele gave a strong selective disadvantage in certain mixtures of gluconate and ribose, in contrast to its selective advantage in gluconate. The selective advantage of gnd\textsuperscript{+} (862) in gluconate decreases very rapidly. If the curve in figure 5 relating selection coefficient to percent gluconate is assumed to be continuous, then it shows a region of selective neutrality at \(\sim 20\%\) ribose, decreases to a selective disadvantage of \(\sim 3.0\%\)/h in 25\% ribose, and returns again almost linearly to selective neutrality in 100\% ribose (fig. 5).

The reversal of selection depending on nutrient composition is not limited to gluconate and ribose. Results of competition experiments in mixtures of gluconate and glucose and of gluconate and succinate are shown in table 3, along with the gluconate-ribose results for comparison. In all three cases, the strain containing
Fig. 4.—Nucleotide and amino acid sequence of relevant portion of \textit{gnd}\(^{+}\)(K12) allele. The sequence of \textit{gnd}\(^{+}\)(811) is identical except for eight nucleotides, indicated by capital letters above the sequence. The sequence of \textit{gnd}\(^{+}\)(862) is identical except for a deletion with breakpoints between each pair of vertical bars. Promoter \(-10\) and \(-35\) sequences are indicated. The downward-pointing arrow at position 583 indicates a transcription initiation site. The lowercase letters and delta (\(\Delta\)) symbol indicate differences from the sequence published by Nasoff et al. (1984). The slash indicating the interval 390–391 shows the position at which the published sequence has a G nucleotide inserted, and the \(\Delta\) at position 426 indicates that the published sequence is missing one member of the pair of T nucleotides at positions 425–426.
FIG. 5.—Estimated selection coefficients and SEs for the gnd⁺(862) allele in chemostats when the limiting nutrient consists of various proportions of gluconate and ribose. Positive selection coefficients favor—and negative ones disfavor—gnd⁺(862). The strains in competition for the mixtures of gluconate and ribose were DD1704 [gnd⁺(862)] and DD1660 [gnd⁺(811)].

* gnd⁺(862) is favored in pure gluconate, disfavored in mixtures containing ≥25% of the alternative carbon source, and selectively neutral when gluconate is absent.

Discussion

We can only speculate on the physiological mechanism of the dramatic selective effects of gnd⁺(862) in mixtures of gluconate and alternative carbon sources. The favorable selective effect of gnd⁺(862) in gluconate medium is expected, as is the selective neutrality in ribose or succinate. However, the unexpected feature is that the
Table 3
Substrate Dependence of Selection

<table>
<thead>
<tr>
<th>ALTERNATIVE CARBON SOURCE (%)</th>
<th>ALTERNATIVE CARBON SOURCE (vs. Gluconate)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ribose</td>
</tr>
<tr>
<td>0</td>
<td>0.038 ± 0.003***</td>
</tr>
<tr>
<td>5</td>
<td>0.034 ± 0.003***</td>
</tr>
<tr>
<td>10</td>
<td>0.025 ± 0.004***</td>
</tr>
<tr>
<td>25</td>
<td>-0.030 ± 0.001***</td>
</tr>
<tr>
<td>50</td>
<td>-0.021 ± 0.002***</td>
</tr>
<tr>
<td>75</td>
<td>-0.011 ± 0.001***</td>
</tr>
<tr>
<td>100</td>
<td>0.001 ± 0.001</td>
</tr>
</tbody>
</table>

Note.—Selection coefficients were estimated in chemostat competition experiments between strains DD1704 [gnd+(862)] and DD1660 [gnd+(811)] when growth rate was limited by gluconate mixed with alternative carbon sources to a total concentration of 0.1 g/liter. Positive entries indicate that DD1704 was favored; negative values indicate that DD1660 was favored. ± Value indicates SE.

*** P < 0.01 (other entries do not differ significantly from 0).

shape of the selection curve is not monotonic. In the transition from 100% gluconate to 75% gluconate, the selection coefficient changes from positive to negative. Whatever the physiological mechanism, this result suggests why a limited activity of 6PGD would be selectively advantageous under natural conditions.

In any event, a dramatic reversal in sign of the selection coefficient results from seemingly minor changes in the growth conditions. Such effects could well be significant in natural populations of Escherichia coli, which appear to be subjected to highly heterogeneous environments, as evidenced by the greater nutritional versatility of strains isolated from free-ranging baboons as compared with those from baboons habitually associated with humans (Routman et al. 1985). Selection favoring alternative alleles in different host environments also appears to be important in maintaining a functional or nonfunctional state of cryptic genes in bacterial populations. For example, E. coli isolates from horses are significantly more likely to be able to utilize cellobiose than are isolates from other sources (Hall and Faunce 1987). The concept of alleles with latent selection potentials that produce different selective effects in different environments has recently been discussed in a general evolutionary context by Stebbins and Hartl (1988).

The deletion occurring in gnd+(862) results in a marked increase in the steady-state level of gnd mRNA. Increased mRNA stability seems unlikely to account for the difference because the mRNA is initiated at the same start site as in wild type. We infer that the increased mRNA level results largely, if not entirely, from increased transcription. At least three mechanisms for the increase may be postulated. First, the juxtaposition of particular sequences may enhance transcription. Second, transcription may be increased because of a change in an unrecognized part of the gnd promoter upstream from the −35 region. Third, it is possible that the deletion eliminates sequences that normally inhibit transcription. Our data are not sufficient to exclude any of these models.

An IS5 element upstream from gnd and occurring only in K12 strains was evidently instrumental in giving rise to the gnd+(862) allele. Although similar deletions may be obtained repeatedly in K12 strains bearing IS5 at this position, we have not found a recurrence among five additional strains recovered from chemostats identical
to that which yielded $gnd^+$ (862). Whether or not the deletion is recurrent in K12 derivatives, identical deletions are not possible in most naturally occurring strains of \textit{E. coli}. The basis for this inference is that approximately two-thirds of natural isolates of \textit{E. coli} lack IS5 sequences (Sawyer et al. 1987), and, among those that do contain IS5, the IS5 position that is upstream from $gnd$ and occupied in K12 is usually unoccupied (Sawyer et al. 1987; Barcak and Wolf 1988). Since the $gnd^+$ (862) allele has a great selective advantage in gluconate medium, non-K12 strains subjected to similar selection pressure may generate other types of mutations that increase $gnd$ activity, perhaps including deletions; but the absence of IS5 in most strains, and the lack of the upstream IS5 in the others, implies that, whatever favorable $gnd$ mutations may arise, they are not very likely to involve IS5.

The inference that the deletion in $gnd^+$ (862) arose through the activity of the upstream IS5 element is based on the positions of the breakpoints. One of the hallmarks of deletions that are mediated by IS elements is that the deletions start precisely at one end of the IS element and terminate at various nonrandom sites (Iida et al. 1983). Although we cannot prove retrospectively that the deletion was IS5 mediated, if the deletion occurred independently of the element, then the location of one random breakpoint precisely at the terminus of IS5 represents a remarkable coincidence.

Examples of insertion elements affecting gene expression are well documented (Hartl et al. 1984; Syvanen 1984); for example, insertions of ISI or IS5 in a region 78–125 nucleotides upstream from the transcription initiation site of $bg/R$ can activate the cryptic gene and allow cells to grow on $\beta$-glucosides (Reynolds et al. 1981, 1986). As another example, plasmids that contain erythromycin-resistance determinants from \textit{Staphylococcus} can be stimulated to express the resistance in \textit{E. coli} by insertions of ISI, IS2, and IS5 (Barany et al. 1982).

The general evolutionary significance of these kinds of effects of IS elements is unclear. Since the number and location of insertion elements differs considerably among natural isolates of \textit{E. coli} (Green et al. 1984; Dykhuizen et al. 1985; Sawyer et al. 1987), favorable effects of IS elements do not result in insertion elements becoming fixed at particular sites in the genome. Since the observed distributions of IS elements fit those expected of selfish DNA (Sawyer et al. 1987), it appears that favorable mutations resulting from IS elements do not occur sufficiently often to materially affect the short-term population dynamics of the elements.

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\section*{Literature Cited}


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