Evolution of Transposable Elements: An IS*10* Insertion Increases Fitness in *Escherichia coli*[1]

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Strains of *Escherichia coli* carrying Tn*10*, a transposon consisting of two IS*10* insertion sequences flanking a segment encoding for a tetracycline-resistance determinant, gain a competitive advantage in chemostat cultures. All Tn*10*-bearing strains that increase in frequency during competition have a new IS*10* insertion that is found in the same location in the genome of those strains. We mapped, by a gradient of transmission, the position of the new IS*10* insertion. We examined 11 isolates whose IS*10* insertion was deleted by recombinational crossing-over, and in all cases the competitive fitness of the isolates was decreased. These results show that the IS*10*-generated insertion increases fitness in chemostat cultures. We named the insertion *fit*::IS*10* and suggest that transposable elements may speed the rate of evolution by promoting nonhomologous recombination between preexisting variations within a genome and thereby generating adaptive variation.

Introduction

Genetic variation must exist for evolution to occur. For asexual organisms, mutation is generally thought to be the sole source of variation. It follows, then, that high mutation rates could be adaptive for asexual organisms if the associated costs of producing deleterious mutations are small. Chemostat studies with asexual populations of the bacterium *Escherichia coli* have demonstrated that mutator (high-mutating) strains have a marked advantage over comparable isogenic but lower-mutating strains (Gibson et al. 1970; Nestmann and Hill 1973; Cox and Gibson 1974). The high- and the low-mutating strains generate beneficial mutations that are phenotypically equivalent, but the mutator strains produce a larger number of such mutations and, hence, evolve faster and gain an advantage (Chao and Cox 1983). Furthermore, this advantage is frequency dependent; the mutator strain is favored only above a starting frequency of $\sim 5 \times 10^{-5}$ because the necessary beneficial mutations cannot be generated in a mutator population below a certain size (Chao and Cox 1983).

Spontaneous mutations (those not induced through artificial mutagenesis) can be generated by two markedly distinct processes. First, they can result from DNA damage or, as in the mutator strains described above, from errors in DNA processing during replication and repair. Second, spontaneous mutations can also arise from insertions of discrete DNA sequences, or transposable elements. Several features characterize transposable elements. They are found in many organisms, they are often present in multiple copies within a genome, they are mobile and can transpose into various sites within a genome through mechanisms independent of homologous re-

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combination, they can alter the expression of genes at or near the insertion sites, and they can promote genetic rearrangements (Calos and Miller 1980; Green 1980; Kleckner 1981; Shapiro 1983). It is not unexpected, then, that bacteria carrying Tn*10*, a composite transposon consisting of two IS*10* insertion sequences flanking an intervening segment carrying a tetracycline-resistance determinant (Foster et al. 1981), acquire a competitive advantage in glucose-limited chemostat cultures (Chao et al. 1983). The resulting advantage, much like that experienced by mutator strains, is frequency dependent, indicating that Tn*10* is acting like a mutator gene. Further supporting this conclusion is the fact that whenever the Tn*10* strain wins, an IS*10* sequence transposes to a new and specific site in the genome. No transpositions are detected when the Tn*10* strain loses (Chao et al. 1983).

The frequency-dependent behavior of Tn*10* populations in chemostat cultures suggests that the new insertion by IS*10* is a specific and beneficial mutation favored by selection in the cultures (Chao et al. 1983). The advantage exhibited by Tn*10*, however, could result from a mechanism independent of transposition, and the new insertion site could be simply an insertion hot spot. In this report we present the results of a study directed at resolving this issue and examining further the evolutionary genetics of Tn*10*. We first mapped the position of the IS*10* insertion in the winning Tn*10* strains. We next deleted the insertion from the winning strains by selecting for recombination of a marker adjacent to the insertion. We then showed that winning strains whose insertion was deleted were at a competitive disadvantage in chemostat competition with strains that still possessed the insertion. We concluded that the insertion increased fitness in chemostat cultures and named this new locus *fit*::IS*10*.

Material and Methods

Strains used in this study are summarized in table 1. Presence or absence of the *fit*::IS*10* and *lac*::Tn*10* markers was confirmed by Southern hybridization blots (see below). These two markers are contained, respectively, within a 3.2- and a 11.6-kb *Pvu*II restriction fragment of the genome (Chao et al. 1983).

Map positions of all reference markers used in the mapping experiments were based on values reported by Bachmann (1983).

Tetrazolium lactose (Miller 1972) and Vogel-Bonner (VB) (Vogel and Bonner 1956) plates were used, respectively, as enriched and minimal media for strain construction and mapping. When required, amino acids, sugars, and antibiotics were added to the media at concentrations specified in Miller (1972) and Maniatis et al. (1982).

P1 transductions, Hfr conversions, and gradient-of-transmission mapping were carried out following standard procedures (Miller 1972).

Total genomic DNA was prepared as described in Hartl et al. (1983). Hybridization blots were carried out by the method of Southern (1975), and colony hybridizations by the method of Maniatis et al. (1982). For hybridization blots, genomic DNA was first digested with *Pvu*II, which does not cut within Tn*10* (Foster et al. 1981). All hybridizations were probed with ^{32}P-labeled pRT61, a ColE1 plasmid containing Tn*10* (Jorgensen et al. 1979).

Chemostat cultures were established and sampled by procedures described in Chao and Cox (1983) and Chao et al. (1983). All cultures were maintained at 37 C and supported with tetracycline-free VB minimal media with limiting 500 μg ml^{-1} glucose. Generation time in these cultures was ~2.8 h.

Table 1
***Escherichia coli* Strains**

Strain	Relevant Markers[a]	Source
χ760	F⁻, *leuB6*, *lacY1*, *galK2*, *trpE38*, *his-208*, *rpsL104*, *ilvA681*	Curtiss and Renshaw (1969)
SM15	F⁻, *xyl-7*, *cysG44*, *argG21*, *lysA22*, *hisG1*, *rpsL104*, Nal^r	Nal^r mutational derivative of strain AT718 (Taylor and Trotter 1967)
W3110	F⁻, prototroph	Gibson et al. (1970)
SM8	F⁻, Str^r	Mutational derivative of W3110
CH1	F⁻, *lac*::Tn*10*	Tn*10*-bearing derivative of W3110 (used by Chao et al. [1983] to start their chemostat cultures)
CH402	F⁻, *lac*::Tn*10*, *fit*::IS*10*	Chemostat-evolved derivative of CH1 (Chao et al. 1983)
SM2	F⁻, *fit*::IS*10*	Lac$^+$ P1 transductant of CH402
SM3	F⁻, *fit*::IS*10*, Spc^r	Mutational derivative of SM2
SM9	F⁻, *fit*::IS*10*, Nal^r	Mutational derivative of SM2
KL96	Hfr, PO44	Low (1973)
SM1	Hfr, PO44, Nal^r	Mutational derivative of KL96
SM4	Hfr, PO44, Nal^r, *fit*::IS*10*, Spc^r	Nal^r Spc^r Hfr recombinant from a mating of SM1 and SM3
KL226	Hfr, PO2A	Low (1973)
CBK070	F⁻, *trpAB*::Tn*5*	Shaw and Berg (1979)
SM10	Hfr, PO2A, *trpAB*::Tn*5*	Kan^r isolate of a P1 transduction of *trpAB*::Tn*5* from CBK070 into KL226
SM11	Hfr, PO2A, *trpAB*::Tn*5*, *fit*::IS*10*, Spc^r	Kan^r Spc^r Hfr recombinant from a mating of SM10 and SM3
SM14	Hfr, PO2A, *trpAB*::Tn*5*, Str^r	Kan^r, Str^r Hfr recombinant from a mating of SM10 and SM8
SM1022	F⁻, Nal^r, Str^r, *fit*::IS*10*	Nal^r Str^r recombinant from a mating of SM14 and SM9
SM1021	F⁻, Nal^r, Str^r	Same as SM1022, but with *fit*::IS*10* crossed out during the acquisition of Str^r
SM1038	F⁻, Nal^r, Str^r, *fit*::IS*10*, *lac*::Tn*10*	Tet^r isolate of a P1 transduction of *lac*::Tn*10* from CH402 to SM1022
SM1039	F⁻, Nal^r, Str^r, *lac*::Tn*10*	Tet^r isolate of a P1 transduction of *lac*::Tn*10* from CH402 to SM1021

[a] Symbols indicate amino acid requirements for leucine (*leu*), tryptophan (*trp*), histidine (*his*), isoleucine-valine (*ilv*), cysteine (*cys*), arginine (*arg*), and lysine (*lys*); inabilities to ferment lactose (*lac*), galactose (*gal*), and xylose (*xyl*); ability to ferment lactose (Lac$^+$); resistances to streptomycin (Str^r and *rpsL104*), spectinomycin (Spc^r), kanamycin (Kan^r), nalidixic acid (Nal^r) and tetracycline (Tet^r); Hfr point of origin (PO); and increased fitness in glucose-limited chemostats (*fit*). Tn*10*, IS*10*, and Tn*5* are transposable elements inserted into the locus preceeding the double colon (::) (Campbell et al. 1979). Tn*10* and Tn*5* carry genes that encode for resistance to tetracycline and kanamycin, respectively (Berg et al. 1975; Foster et al. 1981). The *lac* and *trp* genes at the *lac*::Tn*10* and *trpAB*::Tn*5* loci have been inactivated by the insertions (Shaw and Berg 1979; Chao et al. 1983).

Results

Mapping of *fit*::IS*10*

A genetic locus such as *fit*::IS*10* cannot be mapped by standard Hfr interrupted mating (Miller 1972) because it is not possible to select for the associated phenotypes

(increased fitness in glucose-limited chemostats and presence of an IS*10* sequence) by plating on agar media. Thus, we resorted to mapping through a gradient of transmission in an uninterrupted mating of SM4 (an Hfr donor carrying *fit*::IS*10*) and χ760 (a recipient). We selected for and isolated 107 recombinants that acquired the early entry marker, His^+ (see table 1 for symbol notation), which has a map position of 44 min. The recombinants were then screened for cotransfer of three distal markers—Trp^+, Gal^+, and Lac^+ (map positions of 28, 17, and 8 min, respectively)—and the IS*10* sequence. Screening for IS*10* was carried out by colony hybridization. The Trp^+, Gal^+, and Lac^+ markers were cotransferred to the recipient at frequencies of 14.0%, 4.7%, and 3.7%, respectively, yielding gradients directly proportional to their map distances from His^+. The IS*10* sequence, however, was not acquired by any of the recombinants. Consequently, *fit*::IS*10* was not in the map region between His^+ (44 min) and Lac^+ (8 min).

We next surveyed the map region beyond the Lac^+ marker by mating SM4 and χ760 a second time, selecting for Leu^+ (2 min) and screening for cotransfer of a distal marker, Ilv^+ (85 min), and IS*10*. Of the 108 Leu^+ Str^r recombinants, four (3.7%) were Ilv^+ and one (0.9%) received IS*10*. Since the *E. coli* map is circular and calibrated from 0 to 100 minutes (Bachmann 1983), the resulting gradient of transmission indicated that the IS*10* insertion lay between Ilv^+ (85 min) and His^+ (44 min).

To map the region between Ilv^+ and His^+, we mated SM11 (an Hfr with a point of insertion closer to Ilv^+) with SM15 and then selected for Xyl^+ Nal^r recombinants. A screening of 210 recombinants for five distal markers and IS*10* gave the transmission gradient presented in table 2. The IS*10* insertion was cotransferred 10.7% of the time, a rate that placed the insertion in the 4-min region between Str^s (73 min) and Arg^+ (69 min). A final estimate of 71 min for the map position of *fit*::IS*10* was obtained by assuming a log-linear relationship between cotransfer frequency and map position (Ingraham et al. 1983) and interpolating between Cys^+ and Arg^+.

Table 2
Transfer Gradient in an Uninterrupted Mating of SM11 (Donor) and SM15 (Recipient) with Selection for Xyl^+ Nal^r Recombinants

Marker[a]	Map Position (min)[b]	% Cotransfer of Marker to Recombinants[c]
Xyl^+	80	100.0
Cys^+	74	76.9
Str^s	73	21.8
Arg^+	69	5.6
Lys^+	61	4.6
His^+	44	0
fit::IS*10*	. . .	10.7

[a] Symbols indicate abilities to synthesize cysteine (Cys^+), arginine (Arg^+), lysine (Lys^+), and histidine (His^+); ability to ferment xylose (Xyl^+); and sensitivity to streptomycin (Str^s). See table 1 for other symbol notations.

[b] Bachmann (1983).

[c] Estimates based on 210 Xyl^+ Nal^r recombinants. Nalidixic acid was used to select against the donor.

Effect of *fit*::IS*10* on Fitness

To test whether *fit*::IS*10* was indeed a mutation that increased fitness of bacteria in glucose-limited chemostats (Chao et al. 1983), we constructed isogenic strains with and without the IS*10* insertion and compared their relative fitness in chemostat competition. The required strains were isolated from a mating of SM14 (a Str^r Hfr) and SM9 (a Nal^r recipient carrying *fit*::IS*10*) with selection for Str^r Nal^r recombinants. We then screened the recombinants for loss of the IS*10* insertion by colony hybridization. Since Str^r and *fit*::IS*10* are only 2 map min apart—being located at 73 (Bachmann 1983) and 71 min, respectively—a large percentage (56%) of the recombinants lost the insertion when they acquired the Str^r marker. From the recombinants we isolated two strains: SM1022, which still carried the IS*10* insertion, and SM1021, which lost the insertion (table 1). These two strains, however, could not yet be paired in chemostat competition because they could not be differentiated by any selectable genetic marker. Thus, we transduced *lac*::Tn*10* into SM1021 and SM1022 through selection for the Tn*10*-encoded Tet^r. (We had crossed out *lac*::Tn*10* from CH402, the grandprogenitor of SM9 [see table 1], because the homology between Tn*10* and IS*10* would have masked the presence or absence of the insertion in the colony hybridizations.) The two Tet^r transductants, SM1022 *lac*::Tn*10* and SM1021 *lac*::Tn*10*—renamed SM1038 and SM1039, respectively (table 1)—were, in addition, Lac^- because the Tn*10* insertion had inactivated the *lac* gene (Chao et al. 1983). By using the Lac marker to separate SM1021, SM1022, SM1038, and SM1039, we then tested the four possible Lac^-/Lac^+ pairwise combinations in chemostat competition. The results (figure 1) show clearly that the removal of *fit*::IS*10* caused a decrease in the fitness of SM1021 and SM1039.

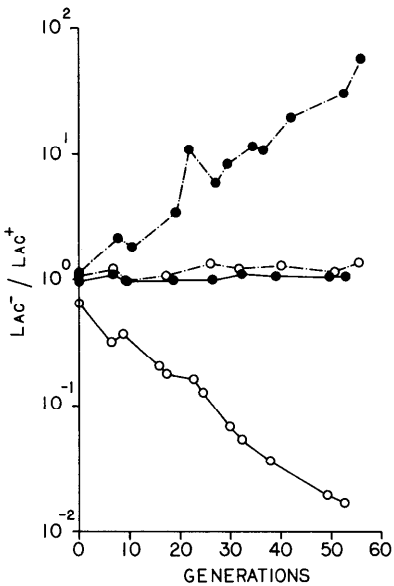

FIG. 1.—Selective advantage of *fit*::IS*10*–bearing strains in chemostat competition. Values on the ordinate axis denote ratios of Lac^-/Lac^+ bacteria on a logarithmic scale. Shown are SM1038 vs SM1021 (● –·– ●); SM1039 vs SM1022 (○ —— ○); SM1038 vs SM1022 (● —— ●); and SM1039 vs SM1021, (○ –·– ○). SM1021 and SM1022 are Lac^+. SM1038 and SM1039 carry *lac*::Tn*10* and are hence Lac^-. SM1022 and SM1038 carry *fit*::IS*10*.

There was no detectable change in the ratio of Lac^-/Lac^+ bacteria in the control SM1038/SM1022 and SM1039/SM1021 chemostats, whereas the Lac^-/Lac^+ ratio increased in the SM1038/SM1021 chemostat and decreased in the reciprocally marked SM1039/SM1022 chemostat.

Replicate Chemostat Experiments

To control for the possibility that the association between *fit*::IS*10* and increased fitness was unique to CH402, the great-grandprogenitor of SM1022, SM1021, SM1038, and SM1039, we examined the behavior of two additional *fit*::IS*10*–bearing mutants. These additional mutants were retrieved from two new chemostat cultures started with different single colonies of CH1, the original progenitor of CH402. By using single colonies we ensured that CH402 and the two new *fit*::IS*10* strains were all independent mutations, unrelated by descent. A set of four competitors, the equivalent of SM1021, SM1022, SM1038, and SM1039, was then derived from each of the new *fit*::IS*10* strains and paired in chemostat competition in combinations comparable to those shown in figure 1.

We compared the results of figure 1 and the two new sets of chemostat cultures by quantifying the fitness difference between the paired strains through a least-squares fit of the trajectories of their population ratios (as in fig. 1) to $\Delta\ln(Lac^-/Lac^+) = S \cdot \Delta G$ (Cox and Gibson 1974), where S denotes fitness difference and G is time in generations. The estimated fitness differences are summarized in table 3 and are grouped into four categories (I–IV) according to the relevant markers of each competitor pair. A single-classification analysis of variance (Sokal and Rolf 1969) found significant ($P < 0.01$) differences between all categories except between III and IV ($0.2 < P < 0.4$) and between I and the absolute values of II ($P > 0.9$). Thus, possession of *fit*::IS*10* always conferred an advantage, irrespective of whether Tn*10* was present in the same strain. Since the population ratios are expressed as number of Lac^- to Lac^+ bacteria, a positive S implies an advantage for the *lac*::Tn*10* strain. The lack of significant difference between category I and the absolute values of category II shows that the opposite signs in two categories were due mainly to the switching of the Tn*10* marker. In fact, Tn*10* behaved in these chemostat cultures as a nearly neutral marker, and the entries in categories III and IV likely were random deviations centered around a mean of 0. Those estimates are of the same magnitude as the resolution of these techniques (Chao and Cox 1983), and a value of 0 is included in the 95% confidence limits of a pooled sample with all the values from categories III and IV (mean ± SEM = $1.4 \times 10^{-3} \pm 1.3 \times 10^{-3}$ generation^{-1}; $n = 6$).

It was still possible, however, that the selective differences seen in figure 1 resulted from the crossing out of a fitness locus tightly linked to *fit*::IS*10* and not from the actual crossing out of the *fit*::IS*10*. In other words, *fit*::IS*10* could still be an insertion hot spot that by chance happened to occur close to a fitness locus. Such a hypothetical fitness locus could have been generated by a pathway independent of IS*10* and then selected for in the original populations studied by Chao et al. (1983). To enable us to place a confidence limit on the minimal linkage distance between *fit*::IS*10* and the hypothetical fitness locus, we isolated eight additional wild-type recombinants from the same mating that produced SM1021 and paired them in competition with SM1038. All eight recombinants lost to SM1038 (category V, table 3).

By pooling categories I and V, we had a combined sample of 11 recombinants that lost their fitness advantage when *fit*::IS*10* was crossed out. The hypothetical fitness

Table 3
Fitness Difference between Strains with and without *fit*::IS*10*

Competitor Category[a]	S (generation^{-1})[b]
I. *fit*::IS*10*, *lac*::Tn*10* vs. wild type	6.5×10^{-2}
	4.2×10^{-2}
	7.0×10^{-2}
II. *lac*::Tn*10* vs. *fit*::IS*10*	-7.0×10^{-2}
	-4.3×10^{-2}
	-6.1×10^{-2}
III. *fit*::IS*10*, *lac*::Tn*10* vs. *fit*::IS*10*	8.8×10^{-4}
	7.6×10^{-3}
	5.1×10^{-4}
IV. *lac*::Tn*10* vs. wild type	3.0×10^{-4}
	2.5×10^{-4}
	-1.3×10^{-3}
V. SM1038 vs. wild type	4.3×10^{-2}
	5.4×10^{-2}
	7.3×10^{-2}
	6.6×10^{-2}
	7.6×10^{-2}
	6.2×10^{-2}
	7.0×10^{-2}
	5.9×10^{-2}

[a] Notation within categories refers to strains equivalent to and including SM1021 (wild type, or strains with *fit*::IS*10* crossed out); SM1038 (*fit*::IS*10*, *lac*::Tn*10*); SM1022 (*fit*::IS*10*); and SM1039 (*lac*::Tn*10*). The eight wild-type strains in category V were additional recombinants from the same mating that produced SM1021 and SM1022 (see Results for details).

[b] S denotes fitness difference (see Results). The first entry in competitor categories I–IV was calculated from the data presented in fig. 1. A positive value of S corresponds to an advantage for the Tn*10*-bearing strain.

locus, however, could be situated either downstream (in the direction of the transfer gradient) or upstream of *fit*::IS*10*. Since the probability (P) that the two markers are not separated by recombination is greater if the hypothetical locus is upstream (de Hann and Verhoef 1966), we chose to determine the more conservative estimate of P by assuming the existence of a downstream configuration. If k is the probability that a crossover event will occur per min of map distance and d is the distance between *fit*::IS*10* and the hypothetical locus, then P is equal to the Poisson null class, or $P = e^{-kd}$ (de Hann and Verhoef 1966; Ingraham et al. 1983). By the 0.05 significance criterion, the lower limit of P for 11 samples is given by $P^{11} = 0.05$, or $P = 0.76 = e^{-kd}$. An estimate of k for the map region near *fit*::IS10 can be obtained from our previous mating of SM14 and SM9 (see above). In that mating 56% of the recombinants acquired both the Str^r marker and the wild-type allele of *fit*::IS*10*—or, for $P = 0.56$ and $d = 3$, $k = 0.2$. With this estimate of k and our lower limit of $P = 0.76$, d = 1.4 min. Thus, if a linked fitness gene existed, it must have been tightly linked to *fit*::IS*10*, with a 0.05 probability that the two were apart by as little as 1.4 min.

Discussion

We have named and mapped *fit*::IS*10*, an IS*10* insertion in *E. coli.* The location of *fit*::IS*10* was ~71 min on the *E. coli* map, and this estimate, determined by a gradient of transmission (Miller 1972), has an error margin of ±5 min (Bachmann and Low 1980). We examined a total of 11 recombinants whose *fit*::IS*10* was crossed out and in all cases there was an associated decrease in relative fitness. Three of the 11 recombinants were derived from independent *fit*::IS*10* mutations, indicating that the association between the insertion and increased fitness arose independently in at least three mutational events. There is a 0.05 probability that the decrease in fitness resulted from the crossing out of a fitness locus linked to *fit*::IS*10* by ≤1.4 min and not from the actual crossing out of *fit*::IS*10*. Considering that the *E. coli* map spans 100 min (Bachmann 1983), however, the probability that the IS*10* sequence in *fit*::IS*10* inserted by chance within 1.4 min of a fitness locus is remote. We conclude, therefore, that *fit*::IS*10* was an IS*10*-generated mutation that was selected for in chemostat cultures and was not an insertion hot spot.

IS*10* could have induced the *fit*::IS*10* mutation by various mechanisms. Since most insertions by transposable elements cause polar mutations by disrupting transcription (Starlinger and Saedler 1976), IS*10* could have (1) silenced a burdensome gene not required by growth in glucose-limited chemostats or (2) inactivated a regulatory protein and turned up an advantageous gene. A small but detectable fraction of *lac*-constitutive mutations result from the insertion of IS*1* into the *lac* repressor (Farabaugh et al. 1978). The facts that Tn*10*-bearing strains were originally observed to have an advantage over Tn*10*-free strains (Chao et al. 1983) and that Tn*10*- or IS*10*-free strains can also generate inactivating mutations do not exclude the possibility that *fit*::IS*10* is an inactivated gene. The Tn*10*-bearing strains could have simply gained an advantage because IS*10* produced more of such mutations, much like the manner in which mutator genes confer an advantage (see Introduction and Chao and Cox [1983]). Alternatively, the IS*10* sequence could have introduced new genetic material with its insertion into *fit*::IS*10*. For example, an IS*2* insertion into *ampC* in *E. coli* created a new promoter and increased by 20-fold the expression of the gene (Jaurin and Normark 1983). Both Tn*10* and IS*10* can provide an outward-directed promoter for transcription of adjacent genes (Ciampi et al. 1982; Simons et al. 1983).

The advantage provided by Tn*10* in chemostat cultures contrasts with that conferred by the transposon Tn*5* (Hartl et al. 1983). The beneficial growth-rate effect exhibited by Tn*5* is physiological and independent of direct transpositions by the transposon. The presence of outward promoter activity in Tn*5* (Ciampi et al. 1982), however, suggests that, under the right selective condition, Tn*5* might also provide an advantage by transposing and introducing a novel promoter. It is possible that outward promoters from different transposons function effectively only with certain genes under specific selective regimens. For example, Tn*10* insertions into *hisG* cause a polar block of downstream genes, whereas similar insertions into ribosomal RNA operons do not (Ciampi et al. 1982). A rho-dependent termination site, believed to be absent in the ribosomal RNA operons, blocks transcription in the *his* locus.

It is noteworthy that we located *fit*::IS*10* at ~71 min and that a previous study (McDonald 1955) mapped a mutation that increased fitness of *E. coli* in glucose-limited minimal medium to the same region of the chromosome, near *rpsL* (73 min). The proximity of these two fitness mutations to the clusters of ribosomal protein and

RNA genes around 72–73 min (Bachmann 1983) raises the possibility that the IS*10* insertion modulated the expression of a ribosomal gene—and hence protein synthesis—to match the requirements of a chemostat environment. Perhaps it is not a coincidence that IS*10* can provide outward promoter function for ribosomal RNA genes and not for other genes such as *his* (Ciampi et al. 1982).

Genetic adaptations in bacteria often result from mutations that change the regulation of genes already present in the organisms. This phenomenon is best illustrated by results of laboratory studies on the evolution of novel metabolic functions in microorganisms (Hall 1983). Since prokaryotic genes can be controlled by several types of regulatory units, e.g., repressors, operators, promoters, terminators, and attenuators (Beckwith and Zipser 1970; Siebenlist et al. 1980; Yanofsky 1981; Holmes et al. 1983), the required regulatory change can be effected by mutations in any one of these units. Although regulatory mutations are undoubtedly generated by processing errors in DNA replication and repair, which are generally point or small mutations, one wonders whether the rate of evolution could be accelerated if the required modulation of a gene under selection were achieved by the replacement of an existing regulatory unit with a second unit that was already present in the genome (see Campbell 1983). Although regulatory units such as promoters, terminators, and attenuators are potentially interchangeable between operons, homologous recombination cannot promote the necessary exchange because the functional DNA sequences in these control units are too short. The functional sequences in promoters, the -35 region and the Pribnow box, are only 6-bp long and separated by a longer stretch of nonhomologous DNA (Siebenlist et al. 1980). Selective pressure for the exchange regulatory units between operons would explain the frequently observed association between IS elements and outward-directed promoters that have no obvious function related to the regulation of genes internal to the element (Ciampi et al. 1982; Simons et al. 1983). The ability of transposable elements to move independently of homologous recombination would make them good vehicles for promoting rearrangements of small regulatory sequences. The IS elements could have acquired the promoters from other bacterial operons or have evolved independently their own outward-directed promoters. Regardless of the origins of the promoters, however, these elements would be providing an added source of variation that is otherwise not accessible to an asexual organism. Since most prokaryotic genomes harbor a large and varied number of IS elements (Calos and Miller 1980), their presence would create a reservoir of promoters with differing strengths. In essence, adaptive variation could arise within an asexual organism through rearrangements of preexisting variation within the genome of the organism. The idea that exchange of nonhomologous genetic material—for instance, translocations—can generate adaptive variation is not new. What we find to be novel in our study is the suggestion that nonhomologous recombination could be an importance source of adaptive variation in an asexual organism such as *E. coli* and that transposable elements were the responsible mechanism.

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