



# Dynamics of insertion sequence elements during experimental evolution of bacteria

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## Abstract

We review the intersection between two areas of microbial evolution that were research foci of Michel Blot. One focus is the behavior of insertion sequence (IS) elements, including their role in promoting the evolutionary adaptation of their hosts. The other focus is experimental evolution, an approach that allows the dynamics of genomic and phenotypic change to be observed in the laboratory. This review shows that IS elements are useful as markers for detecting genomic change over experimental time scales and, moreover, that IS elements generate some of the beneficial mutations that increase organismal fitness.

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## 1. Introduction

Insertion sequence (IS) elements are mobile genetic elements, usually less than 2.5 kb in size, that are widely distributed in the genomes of most bacteria [20]. More than 500 such elements have been identified to date. IS elements are commonly defined as carrying only the genetic information related to their transposition and its regulation, in contrast to transposons that also carry genes involved in other functions (e.g., antibiotic resistance). This review will examine the evolutionary consequences of IS elements and their mutagenic activity, specifically within the context of evolution experiments in the laboratory.

Insertions of IS elements often cause gene inactivation and have strong polar effects [30]. In other cases, however, such insertions can lead to the activation or alteration of the expression of adjacent genes [4,25,29] because some IS elements carry outwardly-directed regulatory sequences including promoters or protein-binding sequences [20]. Besides these local effects, IS elements are also recognized by the recombination machinery of the cell, leading to complex

rearrangements. Depending on their respective orientation, recombination between two homologous IS elements can generate chromosomal inversions or deletions, which are sometimes very large [18,24,31]. The rates of these various IS-mediated events can be quite high, such that IS elements contribute significantly to spontaneous mutagenesis in bacteria [6,12,14,17,27]. A review of the structure of IS elements, and the molecular mechanisms underlying their transposition and recombination activities, can be found elsewhere [20].

From an evolutionary perspective, there are two distinct hypotheses on how IS elements persist in genomes. According to one hypothesis, IS elements are genomic parasites that, on balance, harm their hosts, owing primarily to the increased rate of deleterious mutations that they cause. Such elements are maintained, despite this cost, by replicative transposition to additional genomic sites, including plasmids, coupled with net horizontal transfer into other lineages [2]. The second hypothesis emphasizes that IS elements can also generate occasional beneficial mutations through their transposition and recombination activities. Therefore, IS elements are viewed as important for the adaptive evolution of their hosts, and are maintained by selection for the sometimes useful mutations they produce [1]. Both hypotheses recognize the cost associated with the increased

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load of IS-mediated deleterious mutations, with the difference being whether horizontal transfer of the elements, or selection of beneficial mutations generated by the elements, is the main force responsible for maintaining them in genomes.

The field of evolutionary biology has long been interested in the dynamics of adaptation by natural selection, including the processes that generate the requisite genetic diversity. Evolution experiments in the laboratory provide one way to study these dynamics, and various microbes—including bacteria, yeast, and viruses—have received particular attention because they allow experiments extending for hundreds and even thousands of generations [8]. Microbes offer many advantages for experimental evolution. They are easy to propagate and reproduce quickly, allowing long-term studies with large populations and many replicates. They can be stored indefinitely and later revived, permitting comparison between ancestral and evolved individuals of genotypic and phenotypic features, including their relative fitness based on direct competitions. Environmental variables as well as genetic factors can be manipulated in order to test their effects on evolutionary dynamics and outcomes. The wealth of genomic, biochemical, and physiological information available for many species allows detailed studies of the molecular basis of evolutionary adaptation.

We review here the dynamics and phenotypic effects of IS elements in several evolution experiments using bacteria and, in one case, yeast. These experiments cover a wide range of selective conditions including growth, starvation, and thermal stress. In some of the experiments, IS elements were deliberately chosen to analyze the emergence and maintenance of genetic diversity. In other experiments, mutations were found using various strategies, although some of them were found to have been generated by IS elements. In many cases, IS-mediated mutations were also shown or inferred to be beneficial in the environments in which they were substituted.

## 2. Dynamics of IS elements during 20 000 generations of bacterial growth

### 2.1. IS elements are markers of genetic diversity

The longest-running evolution experiment involves 12 populations of *Escherichia coli* B, all founded from the same ancestral strain [5,15,16]. The populations have been propagated for more than 20 000 generations by daily serial transfers in minimal medium supplemented with glucose. Compared with the ancestor, the competitive fitness of the bacteria increased by about 70% on average, and the average cell volume has more than doubled. The rates of change in both fitness and cell size were faster early in the experiment, and have decelerated as the experiment has continued.

IS elements were used as molecular markers to measure the dynamics and extent of genomic change over the

first 10 000 generations of this evolution experiment [23]. A number of evolved clones were isolated at several time points from two focal populations, designated Ara –1 and Ara +1, and their genomic ‘fingerprints’ were compared to the ancestral strain. The fingerprints were obtained by restriction fragment length polymorphism (RFLP) using IS elements as probes.

This strategy allowed the construction of phylogenetic trees for each of the two focal populations (Fig. 1). Each tree has the unusual feature (relative to most phylogenies) that it is rooted at the true ancestor, and samples come from different points in the population’s evolutionary history. Based on the number of differences between the evolved clones and their ancestor, a genetic distance between the two types was calculated. As expected, the average genetic distance to the ancestor increased significantly with time [23]. Moreover, the IS-associated rate of genomic change in these two populations showed no deceleration over the course of 10 000 generations of evolution. This genomic trajectory stands in contrast to the rates of phenotypic evolution, which decelerated sharply over time. These IS data therefore indicate a discordance between rates of phenotypic and genomic evolution, which has often been suggested but not experimentally demonstrated.

The extent of genetic diversity revealed among contemporary clones was striking. In one population, 11 clones sampled at generation 10 000 all had distinct IS fingerprints. The other population sampled at the same generation showed 10 different fingerprints among 13 clones tested. The temporal pattern of diversity also showed the importance of selection, including evidence for competition among clones carrying different beneficial mutations within the same population. In particular, the phylogenetic trees revealed transient clades that represented a substantial fraction of the population at certain time points, which subsequently went extinct (Fig. 1). These transiently successful, but eventually dead-end, clades are the signature of so-called ‘leapfrog’ events. These events are predicted by mathematical models to occur in large, asexual populations when competing clones independently acquire different beneficial mutations, giving rise to the more general phenomenon of clonal interference [11]. Selective sweeps of beneficial mutations were also suggested by the trunk-like appearance of the phylogenetic trees, along which successive pivotal mutations could be distinguished that eventually spread through the entire population. These pivotal mutations are candidates for being beneficial substitutions. Alternatively, they might have hitchhiked with other beneficial mutations elsewhere in the genome that were not detected by this approach. As a next step, these IS-associated pivotal mutations were characterized at the molecular level.

### 2.2. IS elements can generate beneficial mutations

All of the IS-associated pivotal mutations in the two focal populations through 10 000 generations were analyzed to de-

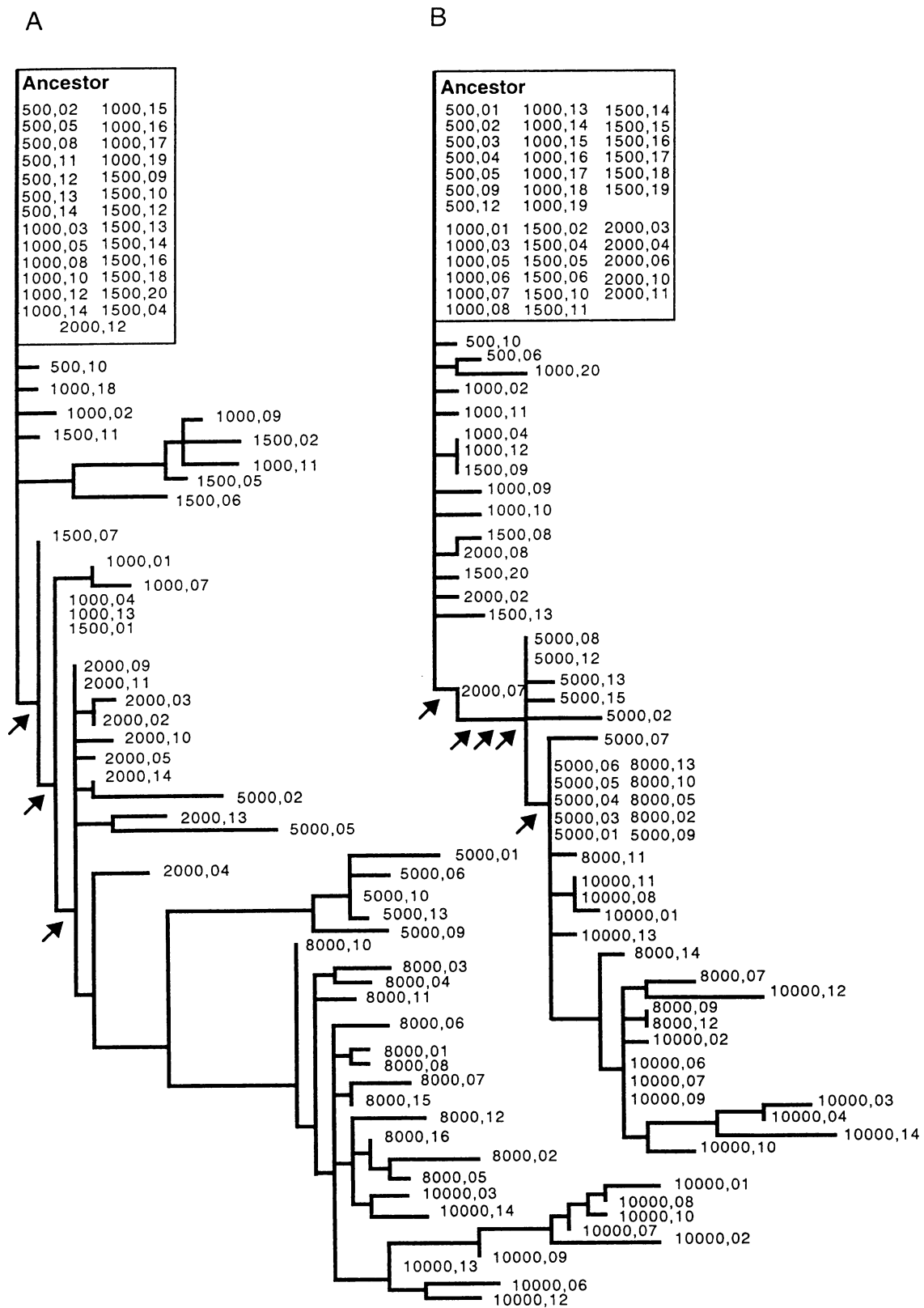


Fig. 1. Phylogenetic trees obtained for two experimental populations of *E. coli*, based on RFLP analysis of the ancestor and numerous evolved clones using IS elements as probes. The figure is reproduced from [23]. Phylogenies were inferred by parsimony from “fingerprints” that consist of the presence or absence of each restriction fragment that hybridized to an IS probe. Notation indicates the generation at which each clone was sampled, followed by an arbitrary number that distinguishes clones from the same time point. Clones shown in the box were identical to the ancestor based on IS fingerprints. Arrows mark some of the pivotal mutations that were shared by all clones in every later sample. (A) Population Ara +1. (B) Population Ara -1. (Copyright 1999 National Academy of Sciences, USA.)

Table 1

Types and genomic sites of pivotal IS-mediated mutations in two populations from the long-term *E. coli* evolution experiment

Mutation	Population	Gene function	First detected at (generation)	Substituted by (generation)
IS150-mediated <i>rbs</i> operon deletion <sup>a</sup>	Ara -1 and Ara +1	Ribose utilization	500	500
<i>pykF</i> ::IS150	Ara -1	Pyruvate kinase I	2000	5000
Inversion between two IS1s (14.1' and 46.9')	Ara -1		5000	5000
Deletion between two IS1s	Ara -1		5000	5000
<i>nadR</i> ::IS150	Ara +1	Repressor of NAD biosynthetic genes	1000	2000
<i>hokB-sokB</i> ::IS150	Ara +1	Homologous to plasmid stabilization systems	1000	2000
IS150 insertion in regulatory region of <i>pbpA</i>	Ara +1	Penicillin-binding protein 2	2000	2000
Inversion between two IS150s (62' and 99.7')	Ara +1		2000	2000
<i>yfcU</i> ::IS150	Ara +1	Unknown	5000	8000
Insertion of IS150 in intergenic region of <i>glcB</i> and <i>yghK</i>	Ara +1	Malate synthase G and unknown	5000	8000

These data are from [31].

<sup>a</sup> Similar deletions of part or all of the *rbs* operon were found in 12 of 12 independently evolved populations [6], including these two focal populations.

termine the underlying molecular events [6,31]. In principle, some of these pivotal changes could have involved mutations in restriction sites used for the fingerprinting, although this possibility was deemed unlikely based on knowledge of the target sizes, mutation rates, and generations elapsed. Indeed, molecular characterization showed that all the pivotal mutations detected by this approach were IS-mediated events; these included insertions, deletions and inversions. These events, including the affected genes, are summarized in Table 1.

An inversion event involving approximately one-third of the *E. coli* chromosome was identified in each focal population, but the two inversions involved different chromosomal regions. In one instance the inversion was bounded by two IS1 elements, and in the other by two IS150 elements. One population also had a deletion that evidently involved recombination between two IS1 elements. The effects of these rearrangements on fitness are not known. Another pair of deletion events was also discovered and characterized, in this case involving similar events in both focal populations. These similar deletions were generated by transposition-recombination events involving IS150 elements, in both cases causing deletion of much or all of the *rbs* operon, which encodes genes required for ribose utilization [6]. Similar IS150-mediated deletions affecting the *rbs* operon were found in all ten other populations, providing a striking example of parallel phenotypic and genomic evolution in the 12 independent lines [6]. These *rbs* deletions had, moreover, been substituted early, reaching almost 100% frequency in all 12 populations within 2000 generations. These rapid and parallel substitutions were shown to be the consequence of two phenomena. First, the *rbs* locus in *E. coli* B is genetically unstable and prone to deletion owing to an adjacent IS150 element. The mutation rate leading to loss of ribose catabolic

function is unusually high at about  $5 \times 10^{-5}$  per cell generation. Second, *rbs* deletion mutations have a selective advantage in the glucose medium used for the experimental evolution. Competition experiments between the ancestor and various isogenic strains with spontaneous or constructed *rbs* deletions revealed a small, but consistent, fitness benefit of these deletions. Elsewhere, it was suggested that antagonistic pleiotropy was the main population-genetic process leading to the ecological specialization of these evolving bacteria to their experimental environment [5]. Antagonistic pleiotropy postulates that losses of function in other environments (causing ecological specialization) are the direct consequence of mutations that are beneficial in the selection environment—as opposed to being caused by the accumulation of neutral or deleterious mutations that drifted or hitchhiked to fixation but do not contribute to adaptation. The demonstration of the fitness advantage in glucose caused by deleting the ribose operon provides a direct confirmation of this evolutionary process.

The most common type of IS-mediated pivotal mutations were transpositions [31]. These mutations, also shared by all descendants in their respective populations (Table 1), affected genes involved in central metabolism (*pykF*, *nadR*), cell-wall synthesis (*pbpA-rodA*), and homologous to plasmid stabilization systems (*hokB-sokB*). Isogenic strains, with and without the mutations, can be constructed in the ancestral and evolved backgrounds. These strains can then be competed to test directly the fitness effect of the corresponding mutation. Preliminary results indicate that at least some of them are indeed beneficial (unpublished data). Moreover, the genes affected by the pivotal IS-mediated insertions in one or the other focal population become interesting can-

didates to screen for parallel, and potentially beneficial, mutations in all the other populations, in a manner similar to that pursued for the IS-mediated deletions of the *rbs* operon.

It should also be emphasized that not all of the IS-mediated mutations were substituted, and not all substitutions were necessarily beneficial, as some may have hitchhiked to fixation. There was a burst of *IS150* activity in one focal population (but not the other), leading to an increase in copy number from 5 in the ancestor to an average of 16.5 in clones sampled at generation 10000. It is quite possible that some of these burst-associated mutations are not beneficial, but instead merely reflect hypermutability possibly caused by derepression of *IS150* transposition. However, it is also clear that at least some of the IS-mediated mutations that were substituted are indeed beneficial.

In summary, IS elements provided a means to measure diversity and quantify genomic change in this experimental evolution. Further characterizing the IS-mediated molecular events, and manipulating the relevant genes, has conclusively demonstrated some beneficial mutations and has also suggested several candidate genes for further study. The identification of beneficial mutations and their phenotypic effects is a central aim of evolutionary genetics, but it is a difficult undertaking. IS elements, in conjunction with evolution experiments, offer a means of tackling this important problem.

### 3. Experimental evolution during prolonged starvation

In the experiment reviewed above, nutrients were renewed each day by serial transfer into fresh medium. By contrast, two other studies have investigated bacterial evolution during severe starvation, and the behavior of IS elements in these populations is described below. The first study focused on *E. coli* that had been stored in agar “stab” cultures for up to 30 years; the second investigated changes in *E. coli* during prolonged “stationary phase” in liquid medium.

#### 3.1. IS-associated genetic diversity in 30-year-old agar stabs

RFLP analyses using IS elements as probes were performed with clones isolated from a stab culture of *E. coli* W3110, which had been stored for about 30 years [21]. This culture consisted of bacteria that had been inoculated as a single clone (from a single colony, derived from a single cell) into an agar-based medium within a glass vial, which was sealed and kept at room temperature for three decades. Although freezers are now widely used for storage of bacterial clones, stab cultures were once the main storage method for bacterial strains; only a short growth period was supposed to occur, presumably limiting the opportunity for genetic change. The existence of such an old stab culture offered Michel Blot and his collaborators an interesting opportunity to test this presumption of genetic constancy.

Substantial genetic diversity was revealed using IS elements: among 118 clones analysed, 68 different hybridization patterns were detected [21]. A phylogeny was constructed that required 174 mutational events in total to explain the observed patterns, with an average of  $\sim 12$  changes leading to each clonal endpoint. Thus, IS elements are important sources of genetic instability in the stab-culture conditions.

One of the challenges this study faced was the lack of the precise ancestor of this old stab culture. However, the dynamics of change were further addressed by performing RFLP analyses on clones isolated from other stabs that had been stored for various periods [22]. The number of IS-related changes showed a positive correlation with the time of storage. It is difficult to calculate a precise mutation rate from these data owing to uncertainty about the rate of cell division (presumably low but not zero) as well as the possible role of selection in eliminating some mutants or favoring others. However, by expressing the mutation rate per unit time, and ignoring any effects of selection, the overall IS-mediated mutation rate in stab-culture conditions was estimated as between  $2 \times 10^{-6}$  and  $9 \times 10^{-6}$  events per cell per hour.

These data clearly show that genetic changes do, in fact, occur in stab cultures. But they do not necessarily mean that the rate of genetic change is faster, per unit time, during starvation than growth, because the average number of changes relative to the ancestor after 30 years in the stab culture was roughly the same as after 4 years (10000 generations) of daily serial transfer and growth [23]. If, as seems likely, the stab culture experienced many fewer generations, then the per-generation IS-mediated rate is probably higher during starvation than growth. In any case, IS elements provided useful markers to find intra-strain polymorphisms and to quantify genomic evolution.

Some of the stab-derived clones were also analyzed phenotypically to determine how they had changed. The majority were auxotrophic, meaning they had lost some metabolic functions. Owing to the difficulty of recreating the environment of an old stab culture, it is difficult to assess whether these strains had merely decayed or, alternatively, had adapted to that environment. However, a few clones showed high fitness under growth conditions.

#### 3.2. Mutants with a growth advantage in stationary phase

Another evolution experiment sought to adapt bacteria to prolonged starvation in liquid medium [34]. Cultures of *E. coli* were grown in rich LB medium for long periods without any renewal of the nutrients. Most cells eventually die, but some remain viable for years [10]. Surviving cells have heritable differences from the ancestor, which persist even after both types are grown under the same conditions. Moreover, the survivors have evidently acquired mutations conferring increased fitness during stationary phase, because they can invade a majority population of ancestral

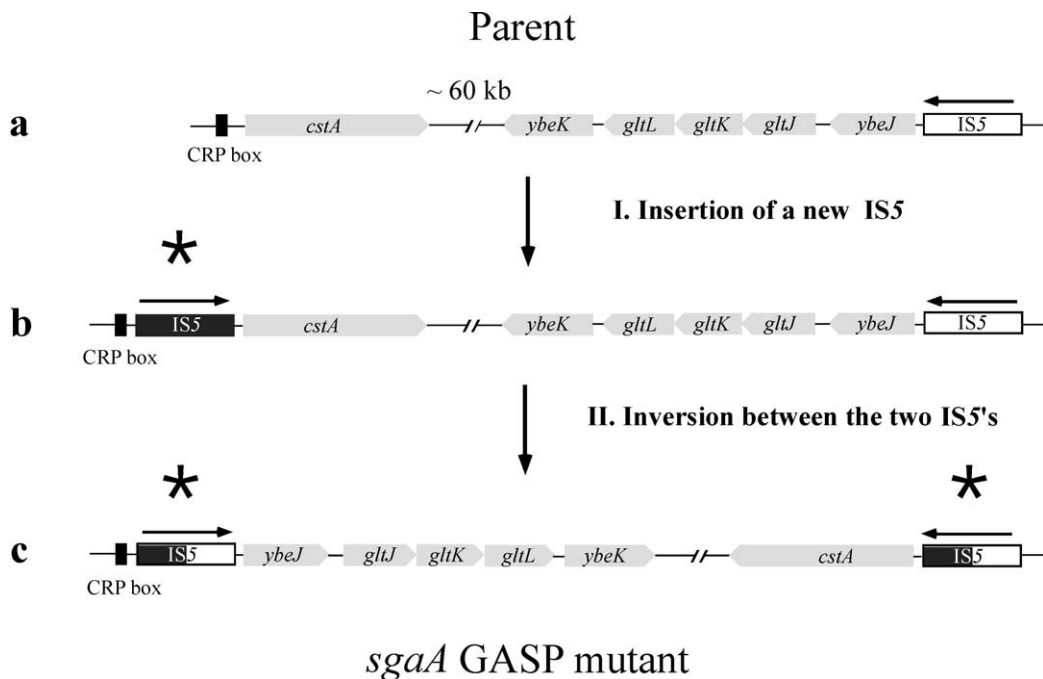


Fig. 2. A complex rearrangement in *E. coli*, involving two IS5 elements, that is beneficial under starvation conditions. Asterisks denote the sites of this two-step genomic rearrangement. (a) In the parent, the *ybeJ-gltJKL-ybeK* operon is ~60 kb away from the *cstA* locus, and they are transcribed in opposite directions. (b) The first step was an insertion of a new IS5 element between the transcriptional promoter and the upstream CRP box of *cstA*. (c) The second step was an inversion between this new IS5 and an existing IS5 located just upstream of the *ybeJ-gltJKL-ybeK* operon. Arrows above the IS5 elements indicate the direction of the transcript.

cells during starvation. This phenotype was called growth advantage in stationary phase, or GASP [34]. Further evolution occurred with increasing time of starvation, involving successive substitutions of GASP mutations [10]. Three different GASP mutations are now known, including two that affect global regulator genes, *rpoS* and *lrp* [34,36]. The third involves IS5 elements, and allowed the discovery of a novel evolutionary mechanism of gene loss involving the expropriation of regulatory sequences [37].

Among its phenotypic effects, this third GASP mutation confers the new ability to grow on aspartate as a sole carbon source [35]. This suggests that the mutant was able to invade the wild-type population by competing more efficiently for amino acids released by dying cells during stationary phase. RFLP analyses using IS elements as probes allowed the genetic characterization of this GASP mutation [37]. A two-step mutation (Fig. 2) involved, first, an insertion of an IS5 element between the promoter and the CRP-binding site of *cstA*, which encodes an oligopeptide permease [32]. A recombination event then occurred between this new IS5 element and an existing IS5 element, located 60 kb away and upstream of an operon, *ybeJ-gltJKL-ybeK*, that encodes an aspartate–glutamate binding protein-dependent ABC transporter [19]. This complex rearrangement caused an inversion that moved the CRP-binding site from *cstA* to the *ybeJ-gltJKL-ybeK* operon (Fig. 2). This inversion led to induction of the operon and inactivated the *cstA* gene. Induction of the evolved operon was shown to occur in stationary phase and was CRP-dependent. Inactivation

of the evolved operon eliminated the GASP phenotype, demonstrating that the IS-mediated mutation was indeed beneficial in stationary phase.

The *cstA* gene encodes an oligopeptide permease, and hence it might also be useful during stationary phase. However, it was inactivated by the inversion. To test the possibility that *cstA* is useful in stationary phase, further competitions were performed using a constructed *cstA*-deletion strain. The competitions showed a positive fitness effect of the ancestral *cstA* gene when it was a minority, and the deletion mutant the majority, during stationary phase. Hence, both the GASP inversion mutant and the wild-type strain, when present as a minority, had a fitness advantage. The two types must therefore occupy distinct ecological niches in the stationary-phase culture environment. When the IS-mediated inversion first appeared, it was of course in the minority. Therefore, it increased because the fitness loss caused by *cstA* inactivation was more than offset by the gain resulting from its new amino-acid growth ability. This new ability reflects induction of the *ybeJ-gltJKL-ybeK* operon, which arose by expropriation of the regulatory sequence of *cstA*. In other words, the IS-mediated inversion created the requisite trade-off in resource utilization via a corresponding reversal of gene expression levels.

Two population-genetic mechanisms that lead to functional losses are well known [5]. One is mutation accumulation, whereby mutations that cause functional losses in other environments are neutral or slightly deleterious in the selection environment; these mutations spread by random

drift or hitchhiking with beneficial mutations at other loci. The second familiar mechanism is antagonistic pleiotropy, in which the functional losses in other environments are caused by the very same mutations that provide the benefit in the selection environment. The expropriation observed in this study is distinct from both these mechanisms because a single event—the inversion—affected two loci, with one effect beneficial and the other deleterious even in the selection environment itself.

This IS-mediated expropriation mechanism may also be important for gene expression after horizontal transfer. Acquisition of new DNA sequences is often not sufficient to express a new function. Expropriation might give bacteria the ability to express newly acquired sequences.

#### 4. Experimental evolution under temperature stress

In this section, and in the next two sections, IS elements were not deliberately used to study genetic diversity and genomic evolution. Nonetheless, mutations involving IS elements were discovered in these experiments.

As part of a study of evolutionary adaptation to different thermal regimes, six populations of *E. coli* B that had previously evolved at a moderate temperature (37 °C) were propagated at a stressful temperature (41.5 °C) for 2000 generations. Genomic DNA from the high-temperature lines and the ancestor was hybridized to high-density arrays to find any duplications or deletions [26]. A total of five duplications and deletions were detected; one line had three such changes, two had a single change each, and three had none. Interestingly, three evolved lines had similar duplications that spanned the same genomic region. This parallelism, coupled with temporal associations with observed fitness gains, suggested the duplications were beneficial in the stressful high-temperature environment. Molecular analysis of these duplications revealed the involvement of IS elements and other types of sequence repeats [26]. Thus, in this study, IS elements were involved in generating duplications of chromosomal regions, and these duplications appear to have been involved in the adaptation of the bacteria to their high-temperature environment.

#### 5. Experimental evolution in chemostats

Bacteria can evolve ecologically important polymorphisms even in simple environments. In one study, twelve populations of *E. coli* were propagated in glucose-limited chemostats for up to 1750 generations [13]. One population was found to have a polymorphism that was maintained by cross-feeding and the resulting frequency-dependent selection [28]. Three distinct clones coexisted at a stable equilibrium when they were mixed together. Their interactions involved differential secretion and uptake of acetate and glycerol, along with competition for glucose [28]. One clone was superior in glucose uptake but secreted acetate,

whereas another clone became efficient in consuming acetate through semi-constitutive overexpression of the *acs* gene, which encodes acetyl CoA synthase, the main enzyme for acetate uptake. It was later discovered that similar polymorphisms had evolved in six of the 12 populations [33]. Further molecular investigation revealed that overexpression of *acs* was caused by two different types of mutation, point mutations and insertions of IS elements. In four populations, identical point mutations were found in the *acs* regulatory region, which increased its similarity to the consensus CRP-binding site [33]. In the other two populations, insertions of an IS3 or an IS30 element occurred upstream of *acs*. IS3 has an outwardly directed promoter at one of its extremities [3]. The insertion of IS3 placed this promoter in the proper orientation to transcribe *acs*, which may explain its up-regulation. IS30 contains an outward facing –35 promoter box at one of its extremities. The IS30 insertion put this –35 box at a favorable distance from the *acs* –10 box, generating a strong promoter sequence and thereby suggesting a mechanism for semi-constitutive expression of the *acs* gene.

In this experiment, IS elements facilitated gene expression through the creation of new upstream regulatory sequences. These changes were sometimes involved in generating stable polymorphisms that were sustained by frequency-dependent selection.

#### 6. Experimental evolution with yeast

Eight lines of *Saccharomyces cerevisiae* were propagated in glucose-limited chemostats for up to 500 generations [7]. Some of them were studied using gene-expression arrays, and evolved clones had transcriptional modifications indicating a shift from fermentative to oxidative metabolism [9]. Chromosomal rearrangements were also studied using array-based comparative genomic hybridizations [7]. Rearrangements were discovered in six lines, including cases of parallel changes [7]. The authors found that most of the observed rearrangements had resulted from translocation events. Characterization of the endpoints of the rearrangements revealed that most were bounded by transposon sequences, either entire Ty elements or their terminal repeats.

This study shows that genomic changes seen in evolution experiments with eukaryotes can be similar to those observed in bacteria. In both groups, mobile elements were directly involved in generating some of the mutations, including complex rearrangements. Regardless of the study organism, the repeatability of independently substituted mutations is evidence for their beneficial effects on fitness in the environment in which they evolved.

#### 7. Conclusions

Transposable elements have sometimes been viewed as genomic parasites that are maintained in genomes only by transposition and horizontal transfer [2]. This view may

well be true some of the time, and it provides an especially attractive hypothesis for the evolutionary emergence of these elements. However, another theory—favored by Michel Blot [1], among others—views these elements as promoting evolution by increasing the variation on which selection can act. While many IS-mediated mutations will be deleterious, this view emphasizes the role of IS elements in generating the rare beneficial mutations that are necessary for adaptive evolution.

In recent years, many biologists have used experimental evolution to investigate microbial adaptation to different environments [8]. Various methods have been used in these studies to quantify genetic diversity, and to identify beneficial mutations. Those studies reviewed here show that the activities of IS elements contribute substantially to the generation of genetic diversity. Moreover, many of the IS-mediated mutations contributed to the adaptation of the microbial populations to their respective environments.

Several more specific conclusions follow:

1. It has been suggested that the activity of IS elements increases during starvation or other stressful conditions [21,22]. Although IS elements were indeed active in evolution experiments performed in stressful environments, they were also active and contributed to adaptation under conditions favorable to growth, including abundant resources and moderate temperature [23]. Thus, IS and other transposable elements are important to genomic evolution and organismal adaptation under non-stressful as well as stressful conditions.
2. IS elements serve as useful markers of genomic evolution, owing to the ease with which IS-mediated mutations can be found and mapped relative to point mutations. Once discovered, the IS mutations become candidate beneficial mutations, especially when similar changes are found in several independently evolved lines. These candidates can be tested by constructing and competing isogenic strains with and without the mutation of interest. Parallel substitutions of IS-mediated mutations were reported in several studies [6,7,26], and some studies performed isogenic competitions to demonstrate the beneficial nature of the mutations [6,37]. Both types of evidence indicate that IS elements are important in producing beneficial mutations.
3. The IS elements generated many different types of mutation in the evolution experiments, including not only insertions but also various rearrangements—deletions, inversions, duplications, and translocations—via recombination between homologous elements. Thus, some beneficial mutations generated by IS activities cannot readily be produced by point mutations [6,7,26,37], although others appear to have similar effects to certain point mutations [33].
4. IS-mediated substitutions affected many gene functions, including central metabolism, sugar transport, cell-

wall synthesis, amino-acid utilization, and regulatory functions. Moreover, IS mutations contributed to a range of evolutionary dynamics including selective sweeps and stable polymorphisms, as well as correlated losses of function via antagonistic pleiotropy and expropriation of regulatory sequences.

In closing, although IS elements may sometimes function as genomic parasites, microbial evolution experiments demonstrate that IS elements can also promote adaptation by generating beneficial mutations.

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