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Differential Spectrum of Mutations That Activate the *Escherichia coli* *bgl* Operon in an *rpoS* Genetic Background

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The *bgl* promoter is silent in wild-type *Escherichia coli* under standard laboratory conditions, and as a result, cells exhibit a β-glucoside-negative (Bgl$^-$) phenotype. Silencing is brought about by negative elements that flank the promoter and include DNA structural elements and sequences that interact with the nucleoid-associated protein H-NS. Mutations that confer a Bgl$^+$ phenotype arise spontaneously at a detectable frequency. Transposition of DNA insertion elements within the regulatory locus, *bglR*, constitutes the major class of activating mutations identified in laboratory cultures. The *rpoS*-encoded σ^S, the stationary-phase sigma factor, is involved in both physiological as well as genetic changes that occur in the cell under stationary-state conditions. In an attempt to see if the *rpoS* status of the cell influences the nature of the mutations that activate the *bgl* promoter, we analyzed spontaneously arising Bgl$^+$ mutants in *rpoS*$^+$ and *rpoS* genetic backgrounds. We show that the spectrum of activating mutations in *rpoS* cells is different from that in *rpoS*$^+$ cells. Unlike *rpoS*$^+$ cells, where insertions in *bglR* are the predominant activating mutations, mutations in *hns* make up the majority in *rpoS* cells. The physiological significance of these differences is discussed in the context of survival of natural populations of *E. coli*.

The *bgl* operon (Fig. 1) is one of four β-glucoside utilization systems present in *Escherichia coli.* It specifies the genes required for the uptake and utilization of the aromatic β-glucosides, salicin and arbutin (22). Wild-type cells are Bgl$^-$, i.e., they cannot utilize arbutin and salicin, at least under laboratory conditions, because the expression of the *bgl* genes is significantly reduced by silencing elements that include DNA structural elements as well as the global repressor, H-NS (19, 21, 25, 31). However, Bgl$^+$ mutants arise spontaneously at a detectable frequency. Most activating mutations isolated in the laboratory are insertion elements (ISs) within the regulatory region, *bglR* (23, 24, 27), that distance or disrupt the negative elements (19, 21, 25). Point mutations in the CRP-binding site (19, 24) and unlinked mutations at loci, such as *hns* (12), *gyrA*, *gyrB* (5), *bglJ* (6), and *leuO* (33), also activate the operon. Once activated, the operon is inducible by salicin and arbutin, and its transcription is regulated by antitermination (20, 26) involving modulation of the binding of the antiterminator to mRNA (13) by phosphorylation (1). The mechanism underlying the silencing of the *bgl* operon in wild-type cells has been extensively studied (3, 21). From an evolutionary viewpoint, retention of the wild-type operon in a silent form, without the structural genes accumulating mutations, is intriguing.

Faced with environmental stress, microbial populations respond by activating inducible systems or, alternatively, exploit genetic processes that can help select for cells better adapted to the new environment. A genetic system that is activated by mutation or recombination may be of particular relevance for enteric bacteria like *E. coli* that are subjected to frequent changes in their immediate environment. The *bgl* operon, with its unusual regulatory mechanism, may represent one such system. The *bgl* genes may be retained in wild-type populations by oscillations between the silent and active states of the operon, with each state providing growth advantage in a different environment (10). Since a majority of the Bgl$^+$ mutants isolated from wild-type (Bgl$^-$) cultures in the laboratory carry insertions of IS*1* and IS*5* in *bglR*, it has been suggested that the transposition of mobile genetic elements could be instrumental in bringing about oscillations between the active and silent states by insertion and precise excision (10). But IS-mediated alternation between active and silent states does not seem possible, at least under laboratory conditions, since in most of the revertants of an IS*1*-activated strain, the operon is permanently inactivated as a consequence of IS*1*-mediated deletions of the structural genes (38).

The *rpoS*-encoded σ^S, the stationary-phase sigma factor, is a key player that enables the cell to survive stress and stasis (18). When cells enter the stationary state, the expression of almost 100 different genes, whose main function is to protect the cell against a variety of stresses (σ^S regulon) is induced or derepressed. This activation is brought about in an RpoS-dependent manner in concert with a combination of one or more global regulators such as Lrp, H-NS, and IHF (11, 14, 37). A number of chromosomal genes, including *rpoS*, affect the transpositions of mobile genetic elements. RpoS has been shown to be required for phage Mu-mediated DNA rearrangements (7, 17). Paradoxically, mutations in *rpoS* also confer a growth advantage in the stationary phase and are the first in a series of genetic changes detected in survivors of prolonged starvation, enabling the efficient scavenging of available nutrients in the environment (40).

Since *rpoS* has been implicated in both physiological as well

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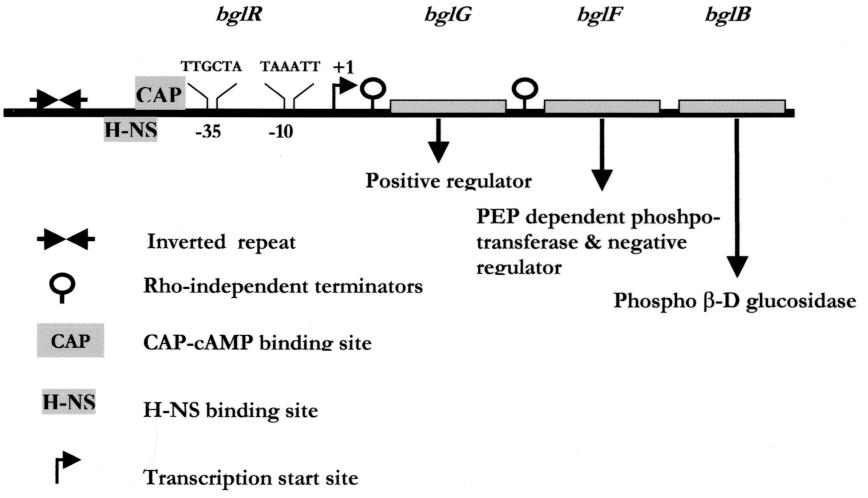

FIG. 1. The *bgl* operon of *E. coli.* The region upstream of the structural genes is termed *bglR*. Activation of the operon occurs predominantly by insertions in *bglR.* Negative regulatory elements, such as the inverted repeat that can extrude into a cruciform and the H-NS binding region, ensure the silencing of the operon in wild-type cells. The catabolite gene activator protein-cyclic AMP (CAP-cAMP) binding site, present upstream of the promoter, overlaps with the H-NS binding site. BglG, which functions as an antiterminator at the two Rho-independent terminators, brings about salicin-inducible transcription of the *bgl* genes upon activation. PEP, phosphoenolpyruvate.

as genetic changes that occur in the cell in stationary-state conditions, we have analyzed spontaneous Bgl^+ mutants of wild-type and *rpoS* cells and show that the spectrum of activating mutations is different in an *rpoS* background. The physiological significance of this observation, in terms of colonization by *E. coli* of its natural habitat, the mammalian large intestine, and secondary habitats, such as soil, is examined.

The *rpoS* mutant strain forms papillae earlier and more frequently than the isogenic *rpoS*$^+$ strain. The wild-type strains RV (*bglR*^{0} *rpoS*$^+$) and SM2 (*bglR*^{0} *rpoS*::Tn*10*) are isogenic (Table 1). Appropriate dilutions of RV and SM2 were plated on MacConkey-salicin plates and subjected to prolonged incubation to allow the colonies to form papillae. We observed that SM2 formed papillae earlier than RV (~24 h for SM2 compared to ~36 h for RV), and there were, on the average, more papillae per colony of SM2. The papillae frequency (measured as the number of papillae/the number of cells) of SM2 was two orders of magnitude higher than that of RV (14.5×10^{-7} for SM2 versus 16.2×10^{-9} for RV). The mean number of Sal^+ colonies arising when overnight cultures of both were plated on minimal salicin plates was also similarly higher for SM2 compared to RV. This indicates that disruption of *rpoS* enhances the mutational activation of *bgl*.

Most *rpoS* papillae do not carry insertions in *bglR*. About 30 papillae from each strain were purified and further analyzed by Southern hybridization or PCR of the *bglR* region to determine the nature of the activating mutation. Wild-type RV, IS*1*-activated RV^+, and IS*5*-activated RVp3 (previously isolated and characterized) were used as controls, and IS*1* or IS*5* insertions in *bglR* were identified as having increased band sizes compared to the wild type (Fig. 2). A remarkable difference was apparent when the activating mutations in the *rpoS*$^+$ and *rpoS* mutant backgrounds were compared (Table 2). In RV, most of the mutants (~80%) showed insertional activation, more than half of which were activated by IS*1*. On the other hand, in SM2 only about 33% (9 of 30 analyzed) showed insertions in *bglR* (Fig. 2). Of these, two were activated by insertions of IS*1* and five were activated by an insertion of IS*5*. In the remaining two (SM2p1.7 and SM2p1.15), the size of the insertion did not match either IS*1* (~0.7 kb) or IS*5* (1.2 kb). On the basis of size and restriction analysis, this insertion was identified as IS*10*. In both of these mutants, the orientation of the IS*10* element was opposite to that of the operon. Thus, unlike in the wild type, in the majority of SM2 papillae (~67%), activation did not involve IS*1* and IS*5* elements and the *bglR* locus remained unaltered with respect to size.

All activating mutations in *rpoS*$^+$ papillae are linked to the *bgl* operon while all noninsertional mutations in the *rpoS* mutant papillae are unlinked. All Bgl^+ mutants of both RV and SM2 that did not carry insertions in *bglR* were subjected to

TABLE 1. Bacterial strains and plasmids used in this study

Strain or plasmid	Description	Source or reference
Bacterial strains		
RV^+	F$^-$ Δ*lacX74 thi bglR11* (*bglR*::IS*1*) (Bgl^+)	A. Wright
RV	F$^-$ Δ*lacX74 thi bglR*^{0} (Bgl^-)	A. Wright
SM2	RV *rpoS*::Tn*10*	This work
MM1	F$^-$ Δ*lacX74 thi bglR*^{0} *tna*::Tn*10* (Bgl^-)	M. Mukerji
AE328	Δ*lacX74 thi bglR11* (*bglR*::IS*1*) *tna*::Tn*10* (Bgl^+)	A. Wright
RVp1-1.31	Papillae from RV (Bgl^+)	This work
SM2p1-1.32	Papillae from SM2 (Bgl^+)	This work
MM1pap1-15	Papillae from MM1 (Bgl^+)	This work
Hfr strains		30
CAG12200	KL16 *zed-3120*::Tn*10*Kan	
CAG12201	KL14 *thi-3178*::Tn*10*Kan	
CAG12202	KL96 *trpB3193*::Tn*10*Kan	
CAG12203	KL208 *zbc-3105*::Tn*10*Kan	
CAG12205	KL228 *zgh-3159*::Tn*10*Kan	
CAG12206	HfrH *nadA3052*::Tn*10*Kan	
Plasmids		
pJL3	*bglJ4, Bam*HI-*Nhe*I fragment in pSK Ap^r	6
pHMG409	*hns, Eco*RI-*Stu*I fragment in pLG339 Kan	8

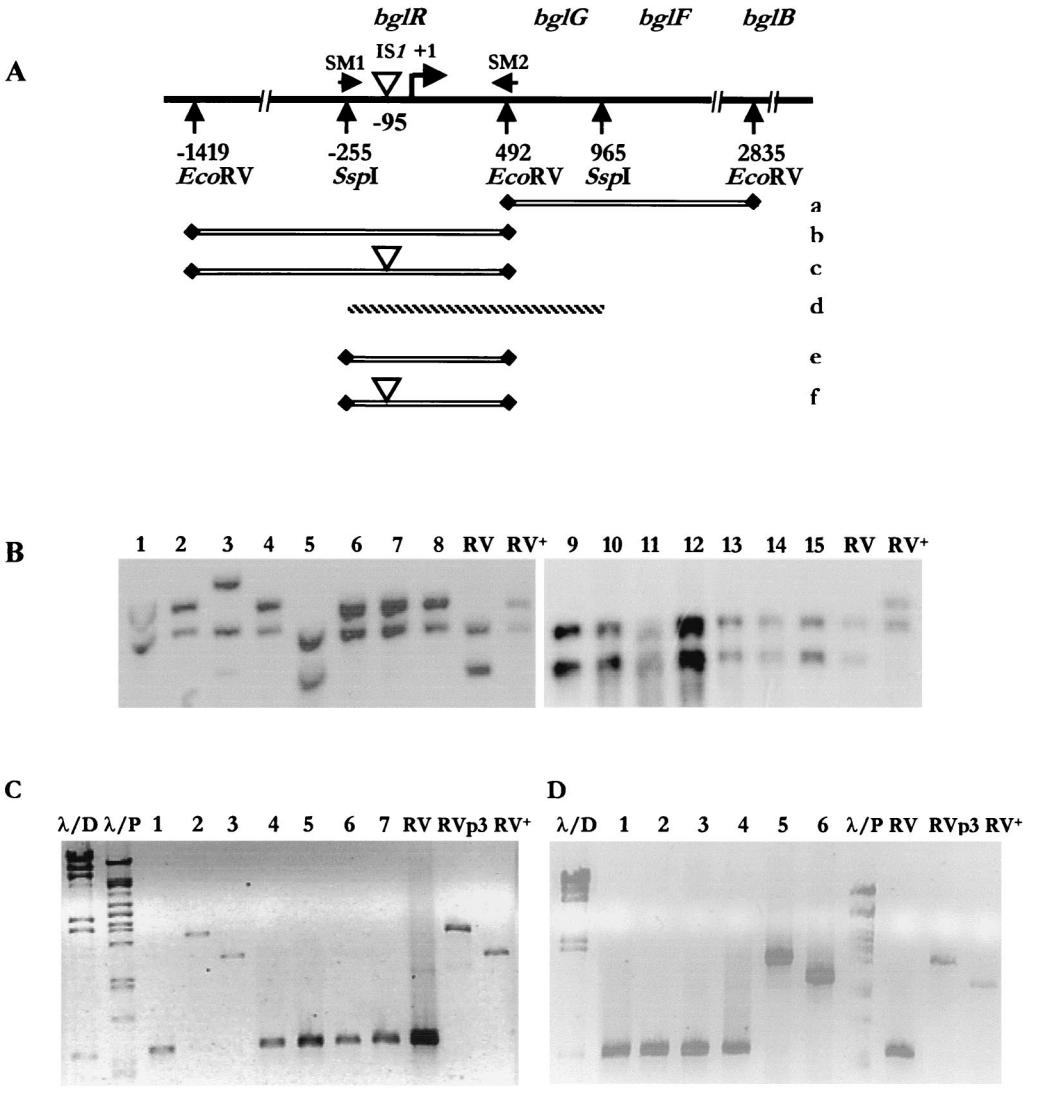

FIG. 2. (A) Schematic representation of the molecular analysis of the *bgl* operon of wild-type cells and Bgl$^+$ mutants. (a to d) Expected bands of genomic DNA digested with *Eco*RV. (d) The 1.2-kb fragment obtained by *Ssp*I digestion of the plasmid carrying the wild-type operon used as a probe. This detects two bands, a 2.3-kb downstream fragment (a) and an upstream fragment (b), which is 1.9 kb in the wild-type and noninsertionally activated operons. (c) This increases in size to 2.6, 3.1, or 3.2 kb upon activation of the operon by the insertion of IS*1*, IS*5*, or IS*10*, respectively. (e and f) Expected bands obtained in PCR analysis. Primers SM1 and SM2 amplify an ~560-bp region in wild-type and noninsertionally activated operons (e). (f) Insertion of IS*1*, IS*5*, or IS*10* results in a larger product, 1.3, 1.8, or 1.9 kb, respectively. (B) Representative Southern analysis of RV (lanes 1 to 8) and SM2 (lanes 9 to 15) papillae. Except for RVp3 (*bglR*::IS*5*) (lane 3) and RVp5 (wild type) (lane 5), all strains show an increase in size in the 1.9-kb band suggestive of IS*1* insertion. All seven SM2 papillae show bands similar to that of the wild-type strain. The wild type, RV, and RV$^+$ (*bglR*::IS*1*) are controls. PCR analysis of representative papillae of SM2 (C) and RV (D). SM2p1.18 is activated by IS*5* (lane 2) and SM2p1.19 is activated by IS*1* (lane 3), whereas SM2p1.17, 1.21, 1.22, 1.23, and 1.24 show products with sizes similar to that of the wild type (lanes 1, 4, 5, 6, and 7, respectively). RVp1.12 is activated by IS*5* (lane 5) and RVp1.13 is activated by IS*1* (lane 6), whereas RVp1.3, 1.6, 1.8, and 1.9 show products with sizes similar to that of the wild type (lanes 1, 2, 3, and 4, respectively). λ/D and λ/P are size markers. The wild type, RV, RV$^+$ (*bglR*::IS*1*), and RVp3 (*bglR*::IS*5*) were used as controls in the PCR analysis.

further analysis. Firstly, they were transduced with a P1 lysate prepared from a strain carrying the wild-type *bgl* operon linked to *recF*::Tn*3* to determine whether the activating mutations are located within the operon. It was seen that all 6 noninsertional mutations in RV were linked to *bgl*, whereas in SM2 all 21 of the mutations were unlinked. Since Southern analysis and PCR of the *bglR* region did not indicate sizes different from those of the wild type (Fig. 2), the RV mutations are probably point mutations or small deletions.

The loci likely to be mutated in the SM2 Sal$^+$ papillae are the other β-glucoside-utilizing operons, namely the *cel* and *asc* operons, both of which are activated by insertions (9, 16), the global repressor *hns* (12), the gyrase genes, *gyrA* and *gyrB* (5), and the recently identified transactivators, *bglJ* (6) and *leuO* (33). To verify that the Sal$^+$ phenotype in the SM2 mutants was a consequence of the activation of the *bgl* operon rather than of other β-glucoside-utilizing operons, *bgl* transcript levels in the wild-type and activated strains were measured. The level

TABLE 2. Spectrum of *bgl*-activating mutations in RV and SM2 papillae[a]

Strain (genotype)	No. of mutations linked to *bgl*				No. of mutations not linked to *bgl*			
	IS*1*	IS*5*	Other insertion	No insertion	*hns*	*bglJ*	*leuO*	Other
RV (*bglR*^{0} *rpoS*$^+$)	15	9	0	6	0	0	0	0
SM2 (*bglR*^{0} *rpoS*::Tn*10*)	2	5	2[b]	0	8	13	0	0

[a] The strains were streaked to isolation on MacConkey-salicin indicator plates and incubated at 37°C until red Sal$^+$ papillae appeared as a result of mutation. Papillae from different colonies were purified and analyzed to determine the nature of the *bgl*-activating mutations. A total of 30 papillae were analyzed in each strain background. The numbers of different types of activating mutations identified in the *rpoS*$^+$ and *rpoS* backgrounds are represented.

[b] Both of these papillae were activated by insertions of IS*10* in *bglR*.

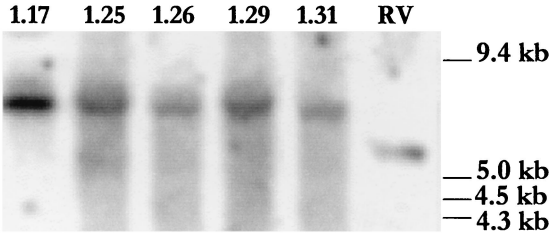

FIG. 4. Southern analysis of the *bglJ* region of representative SM2 mutants showing an insertion of ~1.4 kb. Genomic DNA of the mutants was digested with *Bam*HI and probed with linearized pJL3. RV (Bgl$^-$), which has wild-type *bglJ*, shows a band of ~6 kb while SM2p1.17, 1.25, 1.26, 1.29, and 1.31 (Bgl$^+$) show bands of ~7.5 kb, indicating an insertion.

of *bgl* transcript in the mutants was comparable to that of an IS*1*-activated strain, AE328 (Fig. 3). Further, all mutants were found to be Cel$^-$ (unable to utilize cellobiose), confirming that the Bgl$^+$ phenotype in these strains is independent of the involvement of the *cel* and *asc* operons. Spontaneous mutations that inactivate the gyrase genes, leading to *bgl* activation, are unlikely, as they are expected to be lethal and can be isolated only under specific conditions. When the growth of the mutants was monitored at 37 and 42°C, they showed the same growth rate at 42°C as the wild type, suggesting that they do not harbor conditional mutations in *gyrA* or *gyrB*.

Activating mutations in the *rpoS* papillae map to two loci: *hns* and *bglJ*. The strains derived from SM2 papillae that did not carry an insertion in *bglR* were checked for complementation with the plasmid pHMG409 carrying the wild-type *hns* gene. Of the 21 strains tested, 8 mutants were complemented by *hns*. The activating mutations in the remaining 13 papillae were mapped to a region within 95 to 5 min of the *E. coli* chromosome with the Hfr mapping set (30). Both *bglJ* (99.2 min) and *leuO* (1.8 min) lie in this region. Mutations in these two loci were checked by P1 transduction with strains carrying Tn*5* insertions linked to the two loci. Based on these analyses, the mutations were found to be linked to *bglJ* in all 13 of the strains. The original *bglJ*-activating mutation was an IS*10R* insertion upstream of the gene, resulting in overproduction of the BglJ protein, a putative activator of the *bgl* operon. The SM2-derived *bglJ* strains were subjected to Southern analysis to determine whether the activating mutation was an insertion

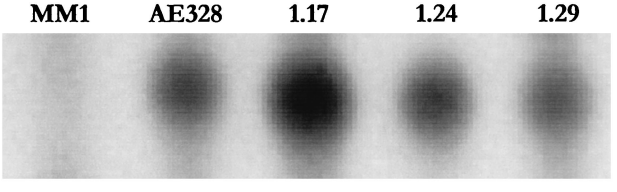

FIG. 3. Detection of *bgl* transcript levels from representative SM2 papillae, SM2p1.17, SM2p1.24, and SM2p1.29 (Bgl$^+$) with the S1 nuclease protection assay as described previously (35). Wild-type MM1 (*bglR*^{0}) and activated AE328 (*bglR*::IS*1*) are the controls. No transcript can be detected in MM1, but the SM2 papillae show transcript levels comparable to those of the insertionally activated strain, indicating that the Sal$^+$ phenotype of the mutants is due to enhanced *bgl* transcription.

in these strains, too. At least five representative strains were found to carry an insertion that, on the basis of size, is likely to be IS*10* (Fig. 4). Although, *leuO* was also identified as a transactivator of *bgl* as a result of insertional activation by mini-Tn*10* Cm leading to overexpression, none of the SM2 papillae appeared to be mutated in *leuO*. Thus, there are differences in the spectrum of the activating mutations in *rpoS*$^+$ and *rpoS* strains.

Most activating mutations in a Tn*10*-carrying *rpoS*$^+$ strain also map to *bglJ*. While RV and SM2 are isogenic at all loci except *rpoS*, there is a major difference between the two: in SM2, a Tn*10* element disrupts *rpoS*. Thus, SM2 is an *rpoS* mutant but also carries a copy of IS*10R* capable of transposition. The differences in activation between the two strains may be a result of the activity of IS*10R*. To verify this, an *rpoS*$^+$ *tna*::Tn*10* Bgl$^-$ strain (MM1) was allowed to form papillae and the papillae were analyzed to determine the nature of the activating mutations. MM1 was found to form papillae at a frequency that is an order of magnitude lower than that of SM2 (~10^{-8}). The majority of the activating mutations were found to be linked to *bglJ* (14 out of 15 papillae). Unlike in SM2, mutations in *hns* were not identified. These results indicate that, though mutations in *bglJ* may be related to the presence of IS*10* in the genome and may occur irrespective of the *rpoS* status, mutations in *hns* are seen only in the *rpoS* mutant genetic background.

The genomes of stationary-state cultures have been shown to be dynamic, and this allows accumulation of changes that improve the fitness of individual cells in the population (40). Since σ^S is the central regulator of cellular changes during starvation, the present study was aimed at analyzing activation of the *bgl* operon in *rpoS*$^+$ and *rpoS* cells. Given that ISs constitute the predominant class of *bgl*-activating mutations, such a study would help towards understanding the role of insertional activation in environments outside the laboratory.

Unlike the *rpoS*$^+$ strain RV, most of the mutations in the *rpoS* strain SM2 (*rpoS*::Tn*10*) are not linked to the *bgl* operon. They fall into two categories. Mutations in *hns* account for about half of them; the remaining mutations are linked to *bglJ*, a putative activator of *bgl*. The nature of the *hns* mutation is not known, but the *bglJ* mutation is an insertion, probably of IS*10*, similar to the original activating mutation (6). The activity of IS*10R* is tightly regulated; its transposition occurs preferentially after DNA replication (4, 29). It is therefore likely

that SM2 appears to form papillae earlier due to IS*10* transposition early during colony growth when cells are actively dividing; other activating mutations (such as IS*1* and IS*5* insertions) occur once cells in the colony stop dividing. This activity of IS*10* is apparently the same in *rpoS*$^+$ and *rpoS* cells. Irrespective of the IS*10* status, the major difference between the two strains is the high frequency of the *hns* mutations seen exclusively in the *rpoS* background. The increase in papillae frequency in SM2 is due to two factors: the presence of IS*10* in the genome and its transposition and increased mutations in *hns* associated with the *rpoS* genetic background.

H-NS is a global inhibitor of gene expression during the exponential phase. Mutations in *hns* pleiotropically increase the expression of various genes, which include *rpoS* itself (39) and a large number of genes belonging to the σ^S regulon (2). Repression of these stress response genes is mediated by H-NS either indirectly via its negative regulation of RpoS or directly by binding to the control regions of these genes. H-NS is believed to have a direct role in silencing the *bgl* promoter (21, 28), and the activating insertions disrupt this interaction. Since the predominant mutations that activate the operon in an *rpoS*$^+$ background are insertions of ISs, the higher frequency of *hns* mutations in the *rpoS* background is suggestive of the fact that selection for these mutations is independent of their positive effect on *bgl*. This is further supported by the observation that four out of five Bgl$^+$ mutants isolated under nonselective conditions from an aged culture of an RpoS-attenuated strain bearing the *rpoS819* allele (40) carried mutations in *hns* (S. Mahadevan and R. Kolter, unpublished data).

Natural microbial populations spend the majority of their lives under starvation stress interspersed with sporadic and short-lived periods of growth when nutrients become available, a feast-and-famine lifestyle (15). While the overall population of stationary-phase *E. coli* cultures may be considered starved, such populations are highly dynamic, and subpopulations arise that consist of mutants with enhanced fitness during starvation. Most of these subpopulations bear a mutation in *rpoS* and consequently have attenuated expression of the σ^S regulon (40). Additional mutations in the *rpoS* background enhance the ability of the cells to scavenge for available nutrients and grow rapidly (41). Thus, rather than maintain a highly resistant nongrowing state, these mutants continue to grow and out compete the wild-type cells in the stationary phase (35). That such population takeovers by *rpoS* cells may be occurring in nature is supported by the allelic variation found in the *rpoS* gene in strains isolated from long-term laboratory cultures as well as from host organisms and secondary environments (32, 34, 36). The balance between the wild-type *rpoS* and its attenuated counterparts will probably depend on the typical amount of time between two feast periods. In nutritionally rich environments, the mortality rate of the wild type would be relatively high and population shifts may be very rapid. In low-nutrient stressful environments, such as minimal media, soil, and water, maintenance and stress resistance functions are of major importance for long-term survival. In such environments, the mortality of the wild type would be low; on the other hand, that of mutants with an attenuated RpoS would be higher since, in these, survival functions would be compromised.

The only other global regulator so far found to accumulate mutations in aged cultures is Lrp (42). Mutations in regulators such as these make global shifts in metabolism and physiology, often with coordinated effect. Alterations in the function of a global regulator would alter several activities and may result in a fitness gain higher than that resulting from altering a single activity. The results reported here suggest that the effect of *hns* mutations in an *rpoS* background may be similar. Given that *hns* normally represses exponential-phase expression of the σ^S regulon, cells with *hns rpoS* double mutations would not only be able to grow rapidly but would also be able to endure stress better. This is consistent with the report that *hns rpoS* double mutants have a faster doubling time than *rpoS* single mutants (2). Though the effect on *bgl* expression may be indirect, the differential spectrum of mutations that activate the *bgl* genes has provided the indication that there is positive selection for *hns* mutations in an *rpoS* background.

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