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A Regulatory Trade-Off as a Source of Strain Variation in the Species *Escherichia coli*†

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There are few existing indications that strain variation in prokaryotic gene regulation is common or has evolutionary advantage. In this study, we report on isolates of *Escherichia coli* with distinct ratios of sigma factors (RpoD, σ^D, or σ^{70} and RpoS or σ^S) that affect transcription initiated by RNA polymerase. Both laboratory *E. coli* K-12 lineages and nondomesticated isolates exhibit strain-specific endogenous levels of RpoS protein. We demonstrate that variation in genome usage underpins intraspecific variability in transcription patterns, resistance to external stresses, and the choice of beneficial mutations under nutrient limitation. Most unexpectedly, RpoS also controlled strain variation with respect to the metabolic capability of bacteria with more than a dozen carbon sources. Strains with higher σ^S levels were more resistant to external stress but metabolized fewer substrates and poorly competed for low concentrations of nutrients. On the other hand, strains with lower σ^S levels had broader nutritional capabilities and better competitive ability with low nutrient concentrations but low resistance to external stress. In other words, RpoS influenced both *r* and *K* strategist functions of bacteria simultaneously. The evolutionary principle driving strain variation is proposed to be a conceptually novel trade-off that we term SPANC (for "self-preservation and nutritional competence"). The availability of multiple SPANC settings potentially broadens the niche occupied by a species consisting of individuals with narrow specialization and reveals an evolutionary advantage offered by polymorphic regulation. Regulatory diversity is likely to be a significant contributor to complexity in a bacterial world in which multiple sigma factors are a universal feature.

The major source of variation in prokaryotes is thought to be the loss or gain of functional genes or elements, such as pathogenicity islands (14, 33). Members of a bacterial species such as *Escherichia coli* have common properties and similar chromosomal organizations, but the species is phenotypically diverse (44). Isolates of *E. coli* exhibit many distinct properties, including distinct growth rates (28) and stress sensitivities (1, 43). Some of the differences are undoubtedly due to loss or gain of genes, but is there also a difference in gene usage or expression between strains? The gene regulatory consistency of bacteria is relatively poorly studied, but it needs to be understood if the full range of bacterial variation is to be established. In this study, we investigated whether strain-specific gene usage is a source of bacterial variation in *E. coli*.

Our starting point for examining this question arose from recent studies of the polymorphism of the RpoS sigma factor in isolates of *E. coli* and *Salmonella* (11, 31). If a central regulator of stress resistance genes (RpoS or σ^S [24, 40]) is not conserved, then how constant is gene usage on a global scale? It is evident from both laboratory studies and the occurrence of *rpoS* mutations in natural populations that regulatory divergence can arise and flourish in particular environments (11). In this study, we found that natural regulatory settings are far from uniform within a species and include a wide range of possibilities.

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A significant level of control over expression of multiple genes in bacteria involves RNA polymerase sigma factors, which partition transcription to different bacterial promoters (13, 17). The concentration of a sigma factor, such as σ^S, controls general stress resistance, starvation survival (16), and gene expression under nutrient limitation (10). In addition, because σ^S competes for a fixed amount of RNA polymerase, the level of σ^S also inversely influences the expression of other σ factor-controlled genes, including housekeeping genes (8, 26). Within this expanding model of cellular control through σ factor competition (20, 21), we investigated whether RpoS protein levels also influenced additional phenotypic and nutritional abilities of various *E. coli* strains. As shown below, an unexpected inverse relationship between stress resistance and nutritional capabilities was found in different strains. Furthermore, a molecular explanation of strain variation can now be offered on the basis of the equally unexpected variation in the endogenous concentration of sigma factors within a species. The numerous implications of these findings for understanding bacterial diversity and evolution are discussed below.

MATERIALS AND METHODS

Strains and strain construction. All bacterial strains used in this study are shown in Table 1. P1 transduction (29) with P1 *cml clr1000* grown on ZK1171 was used to introduce *rpoS*::Tn*10* into BW2952 and MG1655. *lac*$^+$ derivatives of BW2952, BW3709, ZK126, and ZK1171 were made by P1 transduction with P1 *cml clr1000* grown on MG1655.

To study nondomesticated *E. coli* strains, the extensive collection of P. Reeves (Sydney, Australia) was surveyed for *rpoS*-related properties. Forty-one pathogenic and EcoR isolates were screened (34). Of these, only 16 strains were RpoS$^+$ as determined by the glycogen screening test described below. In further phenotypic screening, isolates EcoR38 and EcoR10 and O157:H7 isolate M534 were found to exhibit the range of properties shown by K-12 strains MG1655,

TABLE 1. Strains used in this study

Strain	Relevant genotype	Reference or origin
K-12 *E. coli* strains		
BW2952	MC4100 *malG*::λp*lac*Mu55ϕ(*malG*::*lacZ*)	31
BW3323	MC4100 *rpoS*::Tn*10*	23
BW3708	MG1655 *rpoS*::Tn*10*	This study
BW3709	BW2952 *rpoS*::Tn*10*	This study
BW3726	MC4100 *lac*$^+$	This study
BW3727	BW3323 *lac*$^+$	This study
BW3728	ZK126 *lac*$^+$	This study
BW3729	ZK1171 *lac*$^+$	This study
MC4100	F$^-$*araD139* Δ(*argF-lac*)*U169 rpsL150 deoCl relA1 thiA ptsF25 flb5301 rbsR*	5
MG1655	Supposed fully wild-type strain fully sequenced	2
ZK126	W3110 Δ*lacU169 tna*-2	45
ZK1171	ZK126 *rpoS*::Tn*10*	45
Non-K-12 *E. coli* strains		
BW3736	EcoR38 *rpoS* chemostat isolate	This study
BW3737	M534 *rpoS* chemostat isolate	This study
EcoR38		34[a]
EcoR10		34[a]
M534		Enterohemorrhagic *E. coli* isolate from the State Health Laboratory, Perth, Australia[a]

[a] Obtained from the culture collection of Peter Reeves (Sydney, Australia).

ZK126, and BW2952 and were used for further experiments. An *rpoS* mutation could not be introduced into the P1-resistant non-K-12 strains by transduction, so *rpoS* null mutants of M534 and EcoR38 were isolated directly from chemostat cultures as previously described (31) to obtain strains BW3737 and BW3736, respectively.

Growth medium and culture conditions. The medium used in chemostat cultures was minimal medium A (29). The carbon source in all cases was glucose, which was present at a concentration of 0.02 or 0.04% (wt/vol) in the feed medium in glucose-limiting experiments. For batch cultures and agar plates, glucose or acetate was included at a concentration of 0.2% (wt/vol). Eighty-milliliter chemostat cultures were set up as described previously (31). The dilution rates were set to 0.1 h^{-1} (doubling time, 6.9 h). The culture densities were between 1.9×10^8 and 2.1×10^8 bacteria ml^{-1}.

To assess the metabolism of 95 substrates by the strains in a Biolog GN2 MicroPlate (Oxoid Ltd., Sydney, Australia) (3), the manufacturer's instructions were followed. Positive readings were defined as optical densities at 600 nm of >0.2 after 24 h of incubation.

Detection of *rpoS* status. *rpoS* mutants were distinguished from wild-type strains by staining glycogen in colonies on Luria agar plates. The plates were incubated overnight at 37°C and then left at 4°C for 24 h before they were flooded with concentrated iodine as previously described (31).

***rpoS* amplification and DNA sequencing.** A 1,302-bp fragment containing the *rpoS* gene was amplified from chemostat isolates by PCR by using two external primers, RpoSF1 (5′-CGGACCTTTTATTGTGCACA-3′) and RpoSR1 (5′-TG ATTACCTGAGTGCCTACG-3′), and an internal primer, RpoSI (5′-CTGTTA ACGGCCGAAGAAGA-3′), as previously described (31).

β-Galactosidase and catalase assays. Five-milliliter samples were removed from chemostat cultures, and β-galactosidase activity was measured as described by Miller (29) by using sodium dodecyl sulfate and chloroform-treated cells. KatE/hydroperoxidase II catalase activity was assayed as described by Visick and Clarke (42).

Quantitation of RNA polymerase subunits. Bacteria were harvested from 1-day-old chemostats, extracted, and analyzed by using the standard quantitative immunoblot system (19). Probing was performed with antibodies against purified RpoA, RpoD, or RpoS in parallel with known amounts of purified RNA polymerase subunits. The data presented below are means from three blots of each of two independent samples.

Tolerance to external stress. Assays were conducted with 1-day-old chemostat cultures (31) of each strain. To test acid resistance in rich media, the percentage of survivors was measured after 30 min of exposure to Luria broth acidified to pH 1 with HCl. Bacteria were plated directly onto nutrient agar plates, and dilutions were counted after overnight incubation at 37°C. Survival of bacteria in water was assessed after 15 h of incubation at 25°C.

RESULTS

Strain variation in metabolism and stress resistance. We compared six *E. coli* strains, all *rpoS*$^+$, for metabolism of 95 substrates in a Biolog assay (3). Several strains utilized 47 to 50 substrates, but BW2952 and M534 metabolized only 31 and 24 substrates, respectively (see Table S2 in the supplemental material). To test the possible role of σ^S in metabolism, *rpoS*-defective derivatives of the strains were also assayed. Strikingly, the number of substrates metabolized by M534 and BW2952 greatly increased upon introduction of an *rpoS* mutation (Fig. 1A). The nutritional profiles of the *rpoS* disruption mutants were generally similar. Some individual metabolic differences were found and were probably due to structural gene differences between strains (35), but the results in Fig. 1 suggest that RpoS has a pleiotropic effect on the metabolic capability of certain bacteria. The substrates that were poorly utilized by both BW2952 and M534, whose metabolism was stimulated by an *rpoS* disruption, included D-melibiose, β-methyl-D-glucoside, L-rhamnose, D-sorbitol, acetic acid, D-galacturonic acid, succinic acid, bromosuccinic acid, L-alanine, L-alanyl-glycine, L-asparagine, L-aspartic acid, and DL-α-glycerol phosphate. The complete Biolog results are shown in Table S2 in the supplemental material.

Interestingly, the metabolic capabilities were inversely related to the stress resistance properties of the six strains. Consistent with previous surveys, *E. coli* isolates are not uniformly stress resistant (1, 43) and as shown in Fig. 1B and C, the nutritionally versatile strains, such as MG1655 and EcoR10, were the strains that were most sensitive to stress. Conversely, the nutritionally restricted strains were the most stress resistant. An *rpoS* mutation disrupted resistance to starvation and the osmotic shock that would be experienced during incubation in water, as expected from the established role of RpoS (16). Similarly, resistance to acid was also low in *rpoS* mutants.

Acetate was one of the substrates whose metabolism was

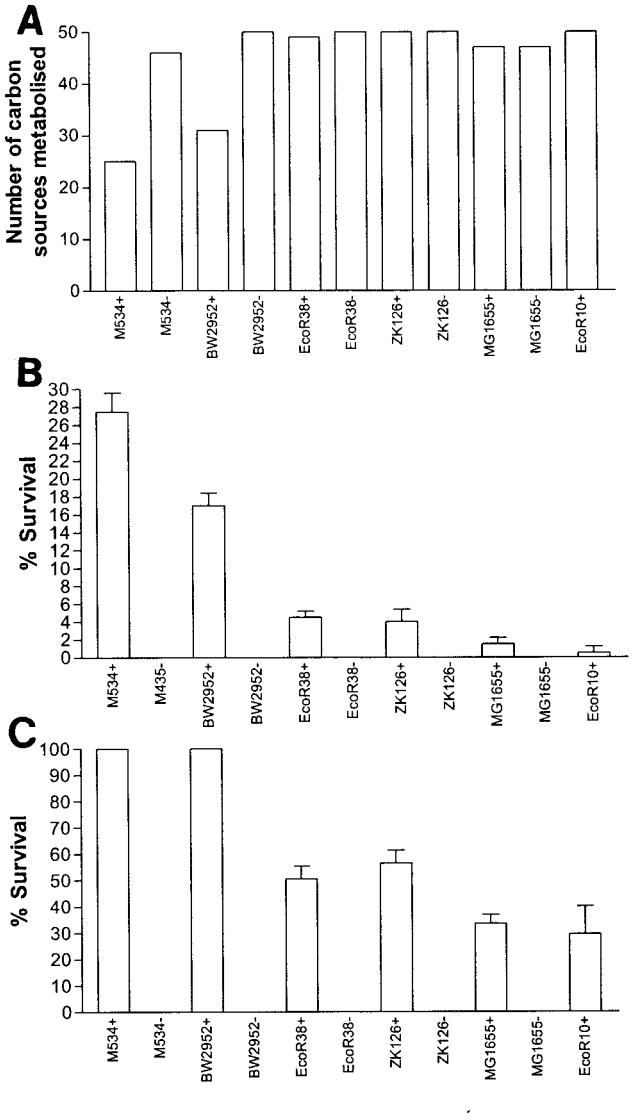

FIG. 1. Strain variation in substrate utilization and the role of RpoS. (A) Numbers of carbon sources metabolized by the strains. A total of 95 substrates were examined with a Biolog GN2 MicroPlate (Oxoid Ltd.) (3). (B) Resistance to exposure to pH 1 for 30 min. (C) Starvation survival after 15 h of incubation in water at 25°C. Assays were conducted with 1-day-old chemostat (31) samples of each strain, and survival was measured by determining viable counts. The error bars indicate the standard deviations based on two replicate experiments. The *rpoS* status of each strain is indicated by a plus sign or a minus sign. The designations of the *rpoS* derivatives of the parental strains are as follows: BW3737 (M534), BW3709 (BW2952), ZK1171 (ZK126), BW3736 (EcoR38), and BW3708 (MG1655).

stimulated by an *rpoS* disruption. A further indication of the role of σ^S in nutrition came from prolonged incubation of the *E. coli* K-12 isolates on acetate plates (Fig. 2). BW2952 showed much poorer growth than MG1655, which is consistent with the Biolog data. Growth of ZK126 was partially impaired on acetate plates. However, after 5 days, individual colonies that grew faster appeared in the BW2952 streak lines on acetate medium. All of these colonies proved to be *rpoS* mutants (data not shown). Growth of a defined *rpoS* derivative of the BW2952 strain, as well as ZK126 (Fig. 2), on acetate was much faster, so the suppression of metabolic capacity by RpoS could be overcome by *rpoS* mutations.

Sigma factor levels in strains of *E. coli*. To test the basis of the differences in metabolic and stress properties among the $RpoS^+$ strains, the endogenous levels of the RNA polymerase components and σ factors (40) were measured in the strains, as shown in Fig. 2 and 3. In quantitating the concentration of the σ^S factor relative to the concentration of a core subunit (RpoA) or the housekeeping-metabolic σ factor (RpoD), it was clear that the RpoD/RpoA ratio was relatively constant (Fig. 3). In contrast, the amount of σ^S varied, and the organisms with a low RpoS/RpoD ratio were more proficient in acetate utilization and metabolism generally. Unexpectedly, the three K-12 strains shown in Fig. 2 differed in the proportion of the sigma factor over a sixfold range during growth on acetate despite having identical *rpoS* sequences (results not shown). The difference in RpoS levels was also not confined to acetate medium, and the concentrations of RpoS protein were markedly different in isolates at identical steady-state growth rates in a glucose-limited chemostat (Fig. 3). Especially interesting was the relationship among stress sensitivity, metabolic capacity, and the endogenous level of RpoS.

Transcriptional effects of distinct RpoS/RpoD ratios. The most likely way that RpoS levels influenced metabolic and stress capabilities was through altered patterns of transcription. The effect of having distinct steady-state RpoS levels in the six isolates was revealed by comparing the expression of housekeeping genes transcribed by using RpoD (σ^D or σ^{70}) with the expression of genes expressed through RpoS or σ^S (Fig. 4). Consistent with the σ^S/σ^D ratios in Fig. 3, quantitation of expression of a σ^D-dependent gene, *lacZ*, showed that there was a trend towards increasing *lacZ* expression with decreasing σ^S in strains, and the highest levels of LacZ were in *rpoS* mutants (Fig. 4A). Conversely, when *katE*, an *rpoS*-dependent gene (30), was examined, the levels of expression were

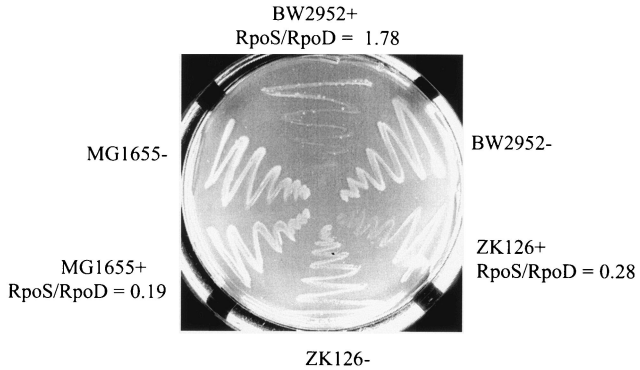

FIG. 2. Strain variation in the growth of *E. coli* K-12 with acetate as the sole carbon source. The *rpoS* status of each *E. coli* K-12 strain is indicated by a plus sign or a minus sign. The designations of the *rpoS* derivatives of the parental strains are as follows: BW3709 (BW2952), ZK1171 (ZK126), and BW3708 (MG1655). The RpoS/RpoD protein ratio in acetate-grown bacteria is indicated for each of the $rpoS^+$ strains; the ratios for the *rpoS* strains were less 0.03. For quantitation of RpoS and RpoD levels in acetate cultures we used the standard quantitative immunoblot system (19).

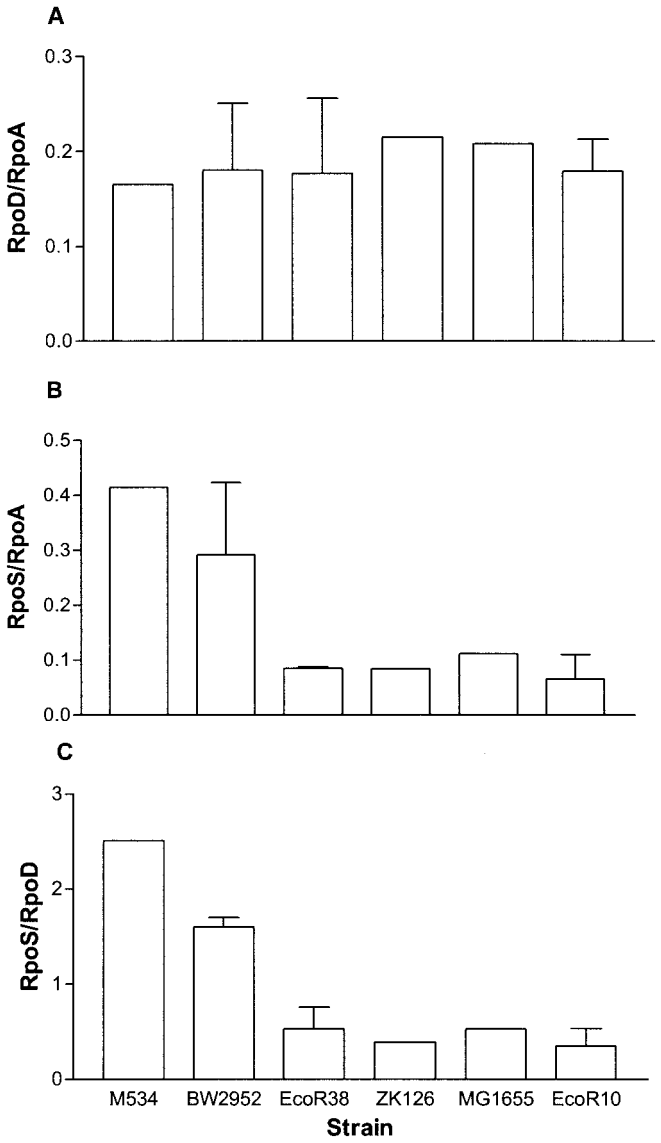

FIG. 3. Strain variation in levels of sigma factors. (A) Quantitation of RpoD relative to core subunit RpoA. (B and C) RpoS/RpoA (B) and RpoS/RpoD (C) ratios of 1-day chemostat samples determined as described by Jishage and Ishihama (19) by using antibodies against purified RpoA, RpoD, or RpoS in parallel with known amounts of purified RNA polymerase subunits. Error bars represent the standard deviations from three blots of each of two independent samples.

highest in the high RpoS strains (Fig. 4B) (EcoR38 was anomalous in not having KatE activity). There was a good correlation between the expression patterns and the stress and metabolism capabilities of the six strains.

Strain variation in mutational adaptation and competitive ability. RpoS levels in different strains of *E. coli* influenced two other bacterial characteristics. First, the mutational adaptation pathway of strains growing under nutrient limitation (10) was initiated differently. Under experimental evolution conditions (32), as shown in Fig. 5, some strains, including strain BW2952 studied previously (11, 31), rapidly accumulated *rpoS* mutations in chemostats under glucose limitation. ZK126 accumulated *rpoS* mutations more slowly, whereas populations of MG1655 did not acquire *rpoS* mutations. Again, there was a good correlation between RpoS and σ^S-dependent transcriptional patterns and the rate of mutation accumulation; the strains with high σ^S levels were under stronger pressure to lose RpoS in a nutrient-stressed situation. These results parallel the acetate mutation selection results shown in Fig. 2.

An important ecological characteristic of bacteria is the ability to compete for low levels of nutrients (9). As shown in Fig. 6, the RpoS status is a major determinant of fitness in a low-nutrient environment. The BW2952 strain with a high level of σ^S was initially outcompeted in a glucose-limited environment compared to MG1655 (Fig. 6A), so not only was the BW2952 strain more restricted in terms of nutritional range, but it also had a lower fitness for glucose. After further growth, the appearance of *rpoS* derivatives in the BW2952 subpopulation increased the competitiveness of the clone, whereas no *rpoS* mutants of the MG1655 bacteria appeared. The proportion of the BW2952 clone continued to increase due to the accumulation of further mutations described elsewhere (32). When competition experiments were started with *rpoS* derivatives of BW2952 and MG1655, there was no initial difference in fitness, suggesting that the two strains had similar metabolic potentials once the constraint imposed by RpoS was removed (Fig. 6).

DISCUSSION

The distinct levels of RpoS in different strains were a major source of phenotypic differences in six strains of *E. coli*. Our results show that even the metabolic profile of bacteria is subject to regulatory variation. This has major implications for microbiology, in which nutrition is often used to type organisms. Our results indicate that the ability to use or not use groups of substrates may be simply a question of global regulation.

Another unexpected conclusion from this study is that a regulatory setting affects both the competitiveness of a bacterium for specific substrates and also its range of substrates. Strains such as EcoR10 and MG1655 are the best specialists for using glucose and also have the broadest nutritional profile. This finding is novel in ecological terms, as generalist and specialist strategies are considered mutually exclusive in ecology (22).

These results also have an impact on our molecular understanding of trade-offs in evolution, which are characterized by the inability of an organism to optimize different traits simultaneously (7, 38). The inverse relationship between nutrition and stress resistance exhibited by bacteria with low and high levels of σ^S is not a nutrition-nutrition trade-off like that between *R* and *k* strategists (25) or a specialist-generalist balance (22), but it is a novel stress protection-nutrition SPANC ("self-preservation and nutritional competence") trade-off. Our results are also consistent with the conclusion that there is no expected trade-off in fitness between adapting to low concentrations of nutrients and adapting to high concentrations of nutrients (41). Transcriptional competition between σ factors (8, 26) and the different RpoS/RpoD levels provide a molecular explanation for the set SPANC balance for different isolates.

Historically, it is important that in gene expression studies

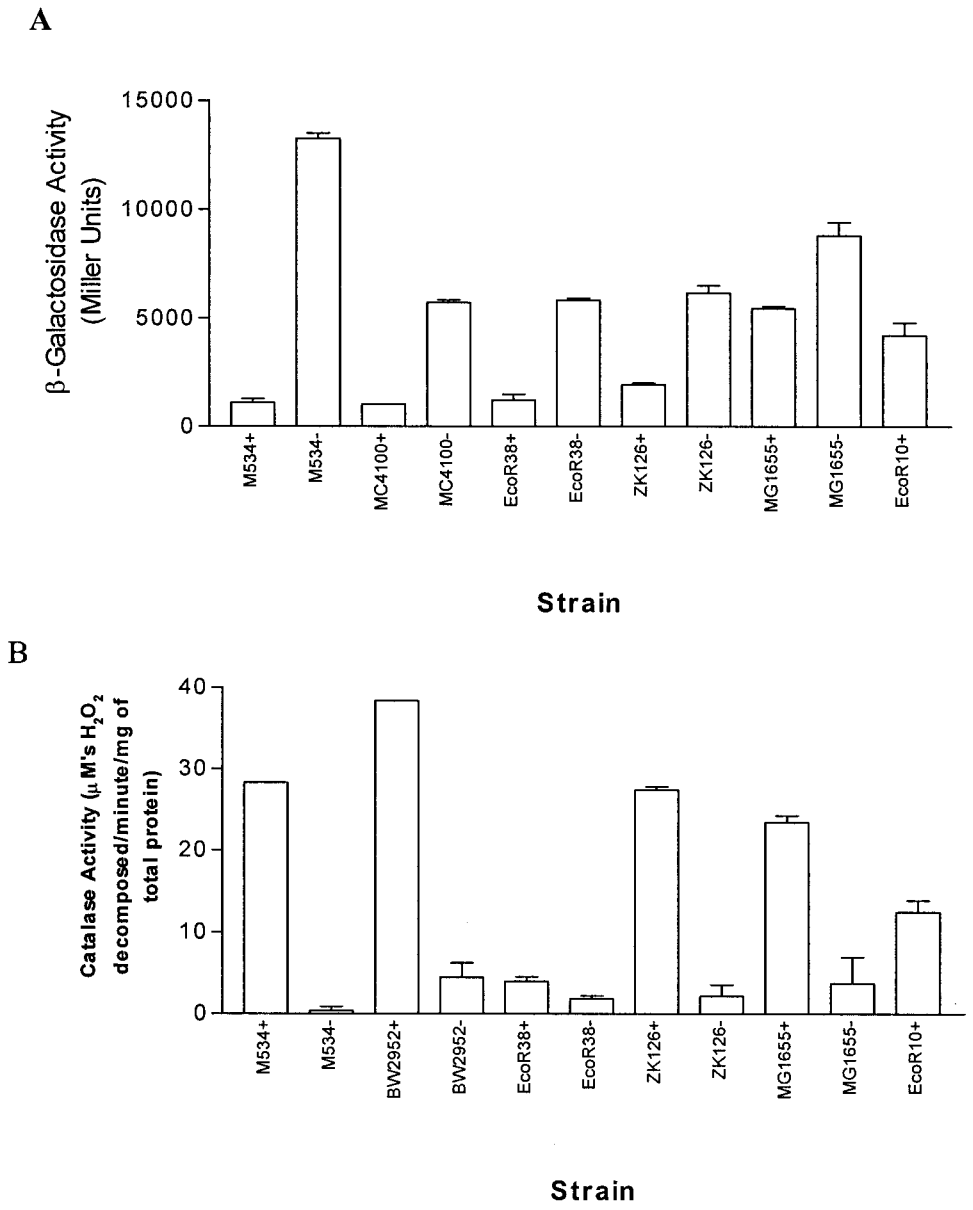

FIG. 4. Strain variation in gene expression. (A) Expression of *lacZ* as determined by quantitating β-galactosidase activity. (B) Specific activity of KatE/hydroperoxidase II (42) of chemostat samples of each strain. The *rpoS* status of each strain is indicated by a plus sign or a minus sign. The designations of the *rpoS* derivatives of the parental strains are as follows: BW3737 (M534), BW3709 (BW2952), ZK1171 (ZK126), BW3736 (EcoR38), and BW3708 (MG1655). The error bars indicate the standard deviations based on two replicates.

with *E. coli* K-12 workers have used numerous genetic backgrounds, including the MG1655, MC4100, and W3110 lineages used here, but our results suggest that RNA polymerase differences need to be considered before strains are interchanged or compared. Indeed, there was a previously noted discrepancy in sigma factor content even within the W3110 lineage (19). It is also relevant that recent results showed that underproduction of RpoD mimics a stringent response (27), which may also partially be the situation in the strains with high σ^S levels. In turn, this may be relevant to the finding that growth rate variation is due to differences in ribosomal function (28), which is in turn subject to stringent control (6). Even more intriguingly, the ratios of other sigma factors may also be subject to trade-offs, because the σ^{54} content of some W3110 strains was also not constant (19).

From our survey, there is insufficient evidence to suggest that particular σ^S levels are associated with particular taxonomic groups or virotypes of *E. coli*. If anything, the evidence points the other way, with a wide range of settings found even within the taxonomic A subgroup (36), including EcoR10 and the three K-12 strains. Still, a more systematic study is needed to test this point. More speculatively, the variation in σ factor levels is likely to be variation that can arise frequently, and it occurred independently in the three K-12 lineages, as can happen during prolonged laboratory storage (19, 39). Adaptation of the SPANC balance is therefore likely to be common in nature.

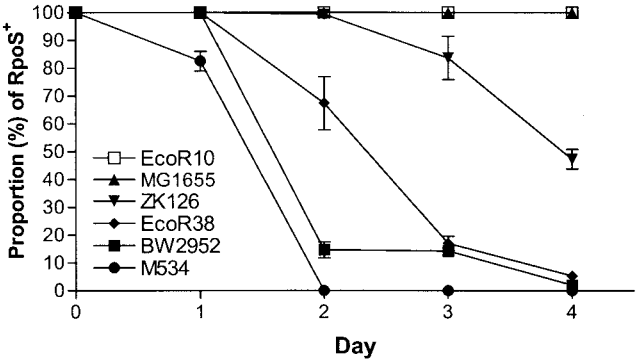

FIG. 5. Selection for loss of RpoS in glucose-limited populations: appearance of *rpoS* mutations in chemostat populations provided with 0.02% glucose at a dilution rate of 0.1 h^{-1}. The proportion of mutants was measured as described by Notley-McRobb et al. (31). The error bars indicate standard deviations based on counts from two or three replicate populations.

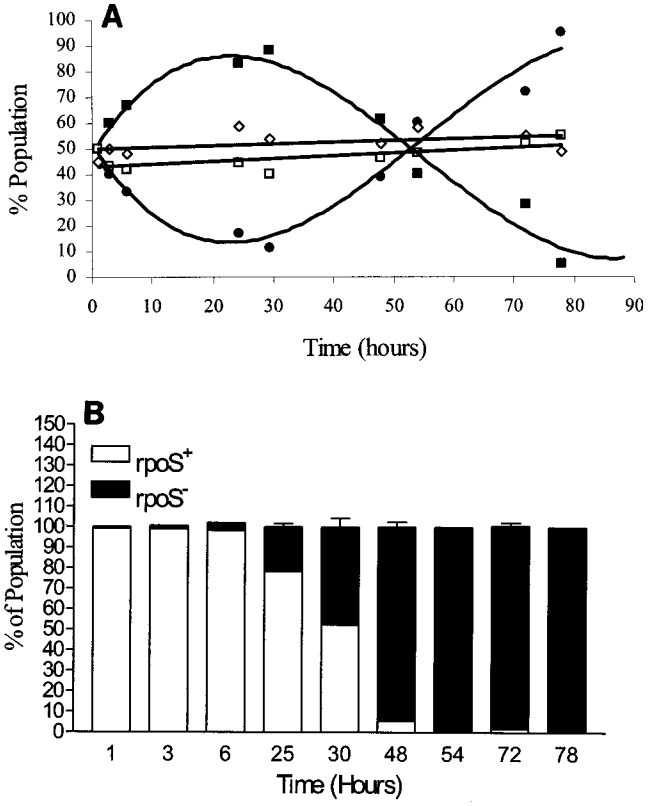

FIG. 6. Role of RpoS in competition for low nutrient concentrations. (A) Chemostat cultures operating at a dilution rate of 0.1 h^{-1} with 0.04% glucose in the feed medium were inoculated with equal proportions of 1-day chemostat-grown cultures of either MG1655 (*rpoS*$^+$) (■) and BW2952 (*rpoS*$^+$) (●) or MG1655 (*rpoS*) (□) and BW2952 (*rpoS*) (◇). The proportion of each strain in the population was determined by plating the appropriate dilution onto Luria agar and selective media. (B) Appearance of *rpoS* mutations in BW2952 in the experiment whose results are shown in panel A. The proportion of RpoS$^+$ cells in the population was determined by staining with iodine. The error bars indicate the standard deviations for three replicate counts.

So far, no explanation for what fixes the discrete but distinct RpoS levels in the different strains is available. At least in the three K-12 strains with identical *rpoS* sequences, the influence on RpoS levels must be extragenic. Complicating matters is the finding that more than one regulatory element may differentiate the strains with low and high RpoS levels because there are numerous, complex inputs for controlling the level of this σ factor in the cell (15). Several regulators control each stage of *rpoS* transcription and translation and σ^S protein stability (18). Detailed investigation of each input is needed to identify the causes of RpoS variation. Intracellular ppGpp was a potential source of variation in RpoS levels, particularly as BW2952 (an MC4100 derivative) has a known *relA1* mutation. However, when ppGpp levels were compared by the method of Rudd et al. (37), there was no correlation between ppGpp levels and RpoS levels. BW2952 had low ppGpp levels but high RpoS levels, whereas M534 had high levels of both. Likewise, the ppGpp level in EcoR10 was lower than the ppGpp level in MG1655, but both strains had low RpoS levels (results not shown). Hence, ppGpp levels are nonuniform in different strains but do not solely explain the RpoS differences observed.

Nevertheless, it is also clear that intragenic changes in *rpoS* can influence all the properties discussed above. Leaky *rpoS* mutations that exhibit partial stress resistance are also known to be selected in particular environments (12, 31); these isolates also show altered transcription patterns and partial increases in metabolic versatility (results not shown). The *rpoS* isolates in population samples (11) also add to the SPANC diversity of bacteria, and *rpoS* mutants are the best-adapted organisms nutritionally (Fig. 1 and 2). Hence, the SPANC setting of members of *E. coli* can be adjusted by both extragenic and intragenic *rpoS* polymorphisms.

In summary, a σ factor protein that is associated with RNA polymerase and central to global gene expression is present at various endogenous levels in a species. Given that multiple σ factors are universal in bacteria, it is highly likely that such variations are common in the prokaryotic world and that variation in genome usage extends to bacteria, as well as to higher organisms (4). The regulatory variation resulting from set levels of RpoS provides a means of broadening the ecological and phenotypic properties of a species. These results suggest that polymorphic regulation is central to understanding the phenotypic properties of bacteria, bacterial strain variation, and the trade-offs between environmentally useful characteristics. Finally, the SPANC trade-off may be a more general kind of evolutionary adaptation that may be important for free-living organisms that encounter nonconstant environments. Speculatively, the availability of multiple SPANC settings can be a considerable advantage to a species by broadening its niche, so individuals with narrow SPANC specialization may fill environments with particular stress-nutrition combinations.

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