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Variation in RNA Polymerase Sigma Subunit Composition within Different Stocks of *Escherichia coli* W3110

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The composition of RNA polymerase σ subunits was analyzed for stock strains of *Escherichia coli* K-12 W3110 in Japan. Heterogeneity was discovered with respect to two σ subunits, σ^{28} (σ^{F}, the *rpoF* gene product) and σ^{38} (σ^{S}, the *rpoS* gene product). Five different types of W3110 were identified: A-type lineages have both σ subunits in intact forms; B-type lineages carry a truncated σ^{38} subunit and an intact σ^{28} subunit; C-type lineages carry an intact σ^{28} subunit but lack a σ^{38} subunit; D-type lineages have only a σ^{38} subunit without a σ^{28} subunit; and E-type stocks lack both σ subunits. All the lineages examined, however, contain the intact forms of σ^{70} (σ^{D}, the *rpoD* gene product) and σ^{54} (σ^{N}, the *rpoN* gene product). As expected from the lack of a σ^{28} subunit, cells of D- and E-type lineages are nonmotile. The truncated form of the σ^{38} subunit in B-type stocks carries two mutations near its N terminus and lacks C-terminal proximal region 4 due to an amber mutation. The failure of C- and E-type W3110 cells to express σ^{38} and that of D- and E-type cells to express σ^{28} were found to be due to defects in transcription even though the respective σ subunit genes remain intact. These findings emphasize the importance of paying attention to possible variations in the genetic background between laboratory stocks originating from the same strain.

The RNA polymerase core enzyme (E) with the subunit structure $\alpha_2\beta\beta'$ carries the catalytic function of RNA polymerization, but promoter-specific transcription initiation requires a σ subunit with promoter recognition activity (5). A number of different molecular species of σ subunits have been identified in bacteria, each recognizing a specific set of promoters (7). Modulation of the promoter selectivity of RNA polymerase by replacement of the σ subunit is an efficient way to alter the global pattern of gene expression in response to changes in environmental conditions (10, 11). At present, seven different molecular species of σ subunits are known to exist in *Escherichia coli.*

During the exponential growth phase, the major σ factor, σ^{70} (the *rpoD* gene product), is responsible for transcription of most of the highly expressed constitutive genes. When *E. coli* culture stops growing due to deprivation of nutrients, the second principal σ subunit, σ^{38} or σ^{S} (the *rpoS* gene product), begins to be synthesized, ultimately reaching 30% of the level of σ^{70} in the early stationary phase (14, 15). The induction of stationary phase-associated phenotypes absolutely requires σ^{38} since some genes for starvation survival are transcribed only by $E\sigma^{38}$ holoenzyme (8, 21). Even during the exponential growth phase, the action of σ^{38} is also required under certain stress conditions such as under high osmolarity (18, 24) or at low temperatures (30).

Motility and chemotaxis are also expedients that bacteria can adapt upon exposure to environmental stress. An ability to move away from stressful areas and into environments favorable for growth is of adaptive value to bacterial cells in nature. The *rpoF* gene product, σ^{28} or σ^{F}, is required for transcription of the regulon involving the genes for flagellar synthesis, flagellar rotation, chemotactic membrane receptors, and chemotactic signal transduction (2, 23). These two alternative σ factors, σ^{38} and σ^{28}, play important roles in bacterial survival in nature.

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Since the number of RNA polymerase core enzyme molecules in *E. coli* is maintained at a constant level characteristic of the rate of cell growth (10, 11), a competition may take place between various σ subunits for binding to the fixed and limited number of core enzymes. This possibility has been supported by measurement of the intracellular concentration of each σ subunit (14, 15). In the course of this study, we realized that the patterns for two σ subunits, σ^{28} and σ^{38}, differ between various stocks of the same W3110 strain, even though *E. coli* W3110 has been widely used for genetic and physiological studies, including the construction of the ordered library of genomic DNA (16) and of the linkage map of the *E. coli* chromosome (4). Since the RNA polymerase σ subunit is a key cellular component determining the global pattern of gene expression, it is possible that additional alterations have accumulated in the genetic background between these stocks to compensate for the differences in transcriptional apparatus. In particular, the responses to various stresses or stimuli may be different between these bacterial stocks because the two σ subunits, σ^{28} and σ^{38}, play important roles in bacterial survival under stress conditions. We therefore decided to carry out a systematic analysis of intrastrain variation in the composition of σ subunits. In this study, we performed a systematic analysis of a number of laboratory stocks of strain W3110, collected from major bacterial genetic laboratories in Japan, with respect to the composition of four σ subunits, σ^{70}, σ^{54}, σ^{38}, and σ^{28}. Results indicate that at least five different lineages of strain W3110 exist in this country, which differ in their content and/or the molecular structure of two stress-response σ subunits, σ^{38} and σ^{28}.

Variation in the σ subunit composition between different stocks of strain W3110. A total of 11 laboratory stocks with the strain name *E. coli* W3110 were provided by a number of laboratories in Japan (Table 1). The strain W3110 was introduced into this country in the late 1950s or early 1960s by first-generation researchers in the field of modern bacterial genetics. The bacterial stocks were maintained as stab cultures on agar slants in tightly sealed glass tubes at room temperature

TABLE 1. Laboratory stocks of *E. coli* W3110 analyzed in this study[a]

Sources (location)	Stock no.	Sigma type[b]
B. Bachmann → Y. Kohara (NIG, Mishima)	1	A
J. Tomizawa (NIH, Tokyo) → H. Ozeki (Kyoto) → H. Inokuchi (Kyoto)	2	A
A. Matsushiro (Osaka) → T. Yura (Kyoto) → C. Wada (Kyoto)	3	A
A. Matsushiro (Osaka) → T. Yura (Kyoto) → C. Wada (Kyoto)	4	A
A. Matsushiro (Osaka) → T. Yura (Kyoto) → K. Ito (Kyoto) → A. Ishihama (NIG, Mishima)	5	C
A. Matsushiro (Osaka) → T. Yura (Kyoto) → R. Okazaki (Nagoya) → Y. Kohara (Nagoya) → A. Nishimura (NIG, Mishima [Kohara Library])	6	B
B. Bachmann → K. Kutsukake (Hiroshima)	7	C
T. Iino (Tokyo) → K. Kutsukake (Hiroshima)	8	C
Y. Hirota (NIG, Mishima) → A. Nishimura (NIG, Mishima)	9	C
M. Cashel → K. Tanaka (Tokyo)	10	D
C. Yanofsky → F. Imamoto (Osaka/Kyoto) → Y. Kano (Kyoto)	11	E

[a] Strain W3110 was a K-12 derivative of *E. coli* constructed in the J. Lederberg laboratory (3). This strain was distributed directly to the Japanese scientists listed in the first column or to American scientists and then transferred to other Japanese scientists from whom the stocks were obtained. Details of the storage conditions and of the inoculation history of stab cultures were not documented, but all these stocks were transferred from stab cultures at room temperature into glycerol stocks at −80°C until the mid-1980s. Stocks 3 and 4 are the permanent and working stocks, respectively, in C. Wada's laboratory. The Kohara library was constructed with a type B stock of strain W3110. NIH, National Institute of Health; NIG, National Institute of Genetics.

[b] For details see Table 2.

for more than 20 years, until the late 1970s to early 1980s, and were then transferred to glycerol stocks frozen at −80°C.

Cells were grown at 37°C under aeration in Luria broth. Cell lysates were prepared at both exponential growth and stationary phases. Using mono-specific polyclonal antibodies raised against purified σ^{70}, σ^{54}, σ^{38}, and σ^{28}, each σ subunit in the cell lysates was determined by the quantitative Western blot method as employed previously (14, 15). All the W3110 stocks examined contained σ^{70} and σ^{54} subunits of apparently intact sizes, as fractionated by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) (Fig. 1). To our surprise, however, marked differences were found in their contents of two other σ subunits, σ^{28} and σ^{38}. From the difference in the immunoblot patterns of these two σ subunits, we classified the bacterial stocks into five types, A, B, C, D, and E (Table 2).

A typical pattern of σ subunits from each type strain is shown in Fig. 1, which indicates that A-type lineage carries both σ^{28} and σ^{38} subunits of intact sizes; B-type lineage contains a truncated form of the σ^{38} subunit with the apparent size of 42 kDa by SDS-PAGE (intact σ^{38} gave 48 kDa under the same running conditions; the truncated form of σ^{38} was originally identified by Kan Tanaka, University of Tokyo, for a B-type lineage of strain W3110) and an intact σ^{28} subunit; C-type lineage contains only the σ^{28} subunit and lacks a detectable level of the σ^{38} subunit; D-type lineage carries only the σ^{38} subunit without the σ^{28} subunit; and E-type bacteria lack both the σ^{28} and σ^{38} subunits. The levels of σ^{70}, σ^{54}, and σ^{28} subunits stayed constant throughout the growth transition from exponential to stationary phases, but σ^{38} was detected only in the stationary-phase cells (15).

Sequence analysis of the truncated σ^{38} subunit in B-type W3110. The B-type W3110 contained a truncated form of σ^{38} subunit which cross-reacted against mono-specific anti-σ^{38} antibodies. In order to determine the structural change(s) in the truncated form of σ^{38} subunit, we cloned the *rpoS* gene from a B-type stock and determined its DNA sequence. As shown in Fig. 2, three single-base changes were detected, which resulted in Leu-to-Phe substitution at position 25, Gln-to-Tyr substitution at position 45, and generation of an amber codon at position 270. Thus, the σ^{38} protein in the B-type *E. coli* W3110 is 269 amino acid residues in length (intact σ^{38} is composed of 330 residues) with a M_r of 30,854 and is almost entirely devoid of region 4, which is highly conserved among σ family proteins and thought to be involved in promoter −35 recognition (7, 22).

The amino acid sequence of σ^{38} displays a high homology to σ^{70} (22), especially in regions 2.3, 2.4, and 4.2, and it has been suggested that it may be a second vegetative σ factor rather than an alternative σ factor. In fact, a number of promoters are

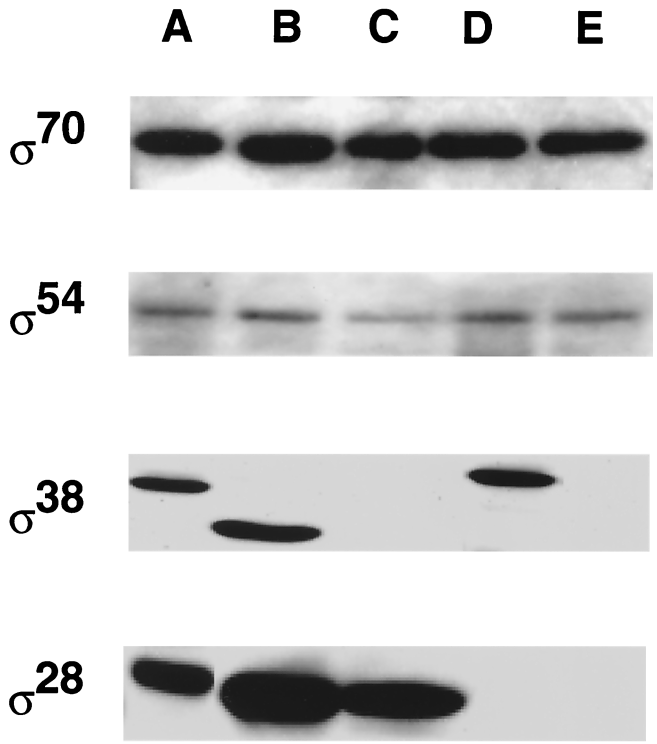

FIG. 1. Content of RNA polymerase σ subunits. *E. coli* A-, B-, C-, D-, and E-type W3110 were grown at 37°C in Luria broth until the early stationary phase. Cell lysates were prepared according to a previously described method (14, 15). The protein concentration of cell lysates was determined by using a protein assay kit (Bio-Rad). The quantitative Western blot was performed for the cell lysates by a previously described method (14, 15), except that the enhanced chemiluminescence reagent system (Amersham) was used for color development. The total proteins used were 1 μg (for σ^{70}) or 10 μg (for σ^{54}, σ^{38}, and σ^{28}).

TABLE 2. Diversity in RNA polymerase σ subunit composition and swarming ability between different stocks of *E. coli* W3110

σ subunit	Lineage of laboratory stocks				
	A	B	C	D	E
σ^{70}	+	+	+	+	+
σ^{54} (σ^{N})	+	+	+	+	+
σ^{38} (σ^{S})	+	+ (truncated)	−	+	−
σ^{28} (σ^{F})	+	+	+	−	−
Swarming ability	+	+	+	−	−

recognized by both $E\sigma^{70}$ and $E\sigma^{38}$ (17, 32, 33). Analysis of chimeric promoters formed from σ^{70}- and σ^{38}-dependent promoters indicates that the specificity of σ^{38} recognition is determined mainly by the −10 sequence, which is recognized by region 2.4 of σ subunits (32). It is possible that the truncated form of σ^{38} lacking region 4 is functional in B-type variant cells.

Expression of the *rpoS* gene in C- and E-type W3110. Type C and type E lineages lacked detectable amounts of σ^{38} subunit. Transcripts of the *rpoS* gene were then analyzed by Northern blot hybridization. As shown in Fig. 3, one clear band was observed when RNA isolated from A-type cells was hybridized with the *rpoS* probe. The size of *rpoS* mRNA detected is in good agreement with the previous length estimation of about 1,600 bases, transcription being driven by a single promoter located within the *nlpD* gene upstream of *rpoS* (19, 31). The *rpoS* transcript in the A-type cells was detected, albeit at different levels, for both the exponential and stationary phases, supporting the concept that in the exponential phase, translational repression operates in the synthesis of σ^{38} (34). In contrast, *rpoS* transcripts from C- and E-type cells were very faint or even absent (Fig. 3A). As an internal control, we checked the same RNA blots with a probe of the *rpoD* gene. As shown in Fig. 3B, one transcript of similar intensity was observed for all three stocks of the strain. These results indicated that the defect in the expression of *rpoS* in C- and E-type cells arises at the transcriptional level.

We then determined the DNA sequence from the *Cla*I site located 1,397 bp upstream of the initiation codon of the *rpoS* gene to the stop codon. No difference was detected, however, in the DNA sequence, at least within the range from 286 bp

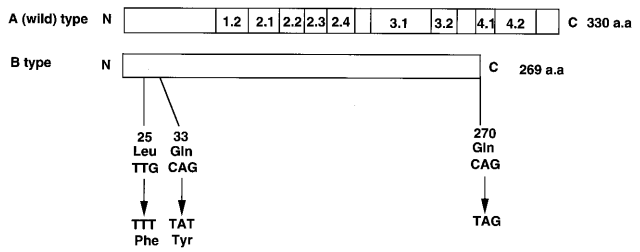

FIG. 2. Nucleotide and amino acid substitutions of the *rpoS* gene in B-type W3110. The *rpoS* gene was PCR amplified from a B-type W3110. PCR primers S5 (with the sequence 5′-GCGAATTCCATATGTTCCGTCAAGGGATCA CG) and S6 (with the sequence 5′-GCGGATCCCTCGAGTTACTCGCGGAA CAGCGCTTC) were synthesized with an Applied Biosystems model 394 DNA synthesizer and purified by PAGE. Template genomic DNA was purified from a lysozyme-SDS-proteinase K-treated cell lysate by phenol-chloroform treatment. PCR amplification was carried out with 1 ng of the genomic DNA, 0.2 μM each primer, and 2.5 U of Ex*Taq* DNA polymerase (Takara Shuzo) in a volume of 100 μl containing 250 μM each deoxynucleoside triphosphate and Ex*Taq* PCR buffer (Takara Shuzo) for 30 cycles, each consisting of 1 min of denaturation at 94°C, 1 min of reannealing at 65°C, and 2 min of DNA synthesis at 72°C. PCR products were digested with *Eco*RI and *Bam*HI and cloned into pUC18 between its *Eco*RI and *Bam*HI sites. Sequencing of the *rpoS* gene was performed by the dideoxy chain termination method (29) by using the Auto Read sequencing kit (Pharmacia) with an A.L.F. DNA sequencer (Pharmacia). Wild-type σ^{38} protein consists of 330 amino acids (a.a.) (31) while the truncated σ^{38} protein in the B-type lineage consists of 269 amino acids. N and C indicate the N and C termini of σ^{38} protein, respectively. The conserved regions of σ^{38} are defined according to Lonetto et al. (22).

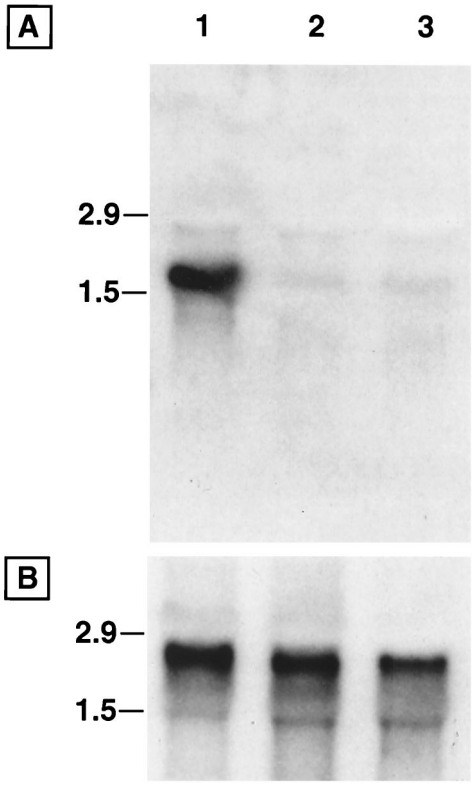

FIG. 3. Northern blot analysis of *rpoS* and *rpoD* transcripts. Total RNAs from A-, C-, and E-type W3110 were analyzed by Northern blotting using specific probes for the *rpoS* (A) and *rpoD* (B) genes. RNA was prepared from bacteria grown exponentially to 30 Klett units according to the method of Aiba et al. (1). Approximately 10 μg of RNA in 20 mM MOPS (morpholinepropanesulfonic acid) buffer (pH 7.0) containing 5 mM sodium acetate, 1 mM EDTA, 17.5% formaldehyde, and 50% formamide was heated for 15 min at 65°C and immediately loaded onto a 1% agarose gel containing 18% formaldehyde and MOPS buffer. After electrophoresis at 100 V, RNA in the gel was transferred to a Hybond-N Plus membrane (Amersham) overnight in 20× SSPE (20× SSPE is 3 M NaCl, 173 mM NaH_2PO_4, 25 mM EDTA). After baking the filter for 120 min at 80°C, hybridization was performed by first soaking the blot for 3 h in a prehybridization solution (5× SSPE, 50% formamide, 5× Denhardt's solution, 0.5% SDS, 0.1 mg of sheared salmon sperm DNA per ml) at 42°C. After adding the probe, hybridization was carried out for 16 h at the prehybridization temperature. The blot was washed twice in 2× SSPE containing 0.1% SDS at room temperature for 10 min, twice in 1× SSPE containing 0.1% SDS at 65°C for 20 min, and once in 0.1× SSPE–0.1% SDS at 65°C for 20 min and was then autoradiographed. Lane 1, A type; lane 2, C type; lane 3, E type. The migration positions of ribosomal RNAs (1.5 and 2.9 kb) are shown on the left. For analysis of *rpoS* transcript, a probe of 1,029 bp of DNA corresponding to the coding region of *rpoS* was prepared by PCR by using S5 and S6 primers and A-type W3110 DNA as a template. For analysis of *rpoF* RNA, a 722-bp fragment covering the coding region of *rpoF* was prepared by PCR with FA1 and FA2 primers for use as a probe. For analysis of *flhD* transcript, a 1,205-bp fragment covering the coding region of *flhD* was PCR amplified with FD1 and FD2 primers for use as a probe. On the other hand, a probe for *rpoD* transcript analysis was prepared by digesting plasmid pGEMD (9) with *Mlu*I and *Pst*I to obtain a 667-bp fragment covering the internal portion of the *rpoD* gene. All the DNA probes were gel purified (29) and labelled by random priming with Megaprime DNA labelling systems (Amersham) and [α-^{32}P]dCTP (Amersham). Labelled probes were separated from unincorporated nucleotides by using SUPREC-02 (Takara Shuzo).

upstream from the initiation codon to the stop codon of *rpoS* (data not shown). Several candidates have been suggested as signals for activation of *rpoS* transcription. For example, a gradual reduction in growth rate stimulates *rpoS* transcription (21), the cyclic AMP (cAMP) receptor protein-cAMP complex represses it (18), but guanosine 3′, 5′ bispyrophosphate (ppGpp) activates *rpoS* transcription (6, 19). It has not yet been determined how the transcription of *rpoS* is blocked in these cell lines.

The expression of *rpoS* is, however, under exceedingly complex regulation, involving a number of regulatory signal molecules and operating at the levels of transcriptional and post-transcriptional regulation as well as stability control of the protein (18, 34). Thus, the possibility cannot yet be excluded that the *rpoS* transcript in the type C and type E lineages is extremely unstable.

Expression of the *rpoF* gene in D- and E-type W3110. Both D- and E-type bacteria lacked the σ^{28} subunit, which is required for the expression of flagellar genes. In agreement with this finding, both stocks are nonmotile as analyzed by the plate assay for motility (data not shown).

In order to investigate whether the D- and E-type lineages carry the gene encoding intact σ^{28} protein, we cloned the *rpoF* gene from these cells and sequenced DNA from the *Stu*I site located 253 bp upstream of the initiation codon of the *rpoF* gene to the stop codon. The sequences were completely identical with that previously reported (25) except for two single-base changes at positions −130 and −188 bp upstream from the initiation codon for σ^{28} translation (data not shown). For determination of the defective step of *rpoF* expression in these bacteria, we then analyzed transcripts of not only *rpoF* but also *flhDC*, which encode a class I transcription factor for expression of stage I genes of the flagellar regulon, including *rpoF* (20). Northern blot analysis with a *rpoF* probe, shown in Fig. 4A, indicates two transcripts for RNA isolated from A-type cells. The *rpoF* gene maps in the *fliAZY* operon (*fliA* has been renamed *rpoF*), which gives a transcript of approximately 2,270 bases (25). The upper band, migrating faster than the 2.9-kbp marker, might be a complete transcript of this operon, while the lower, stronger and more smeared band detected below the 1.5-kbp marker might be a product terminated after the first one or two genes. In contrast to the A-type pattern, virtually no transcript of the *rpoF* gene was detected in RNA from D- and E-type bacteria. Moreover, no detectable transcript of *flhD* was observed in D- and E-type samples (Fig. 4B), even though one clear band of similar intensity was observed for all three samples when the same RNA blot was hybridized with a probe of the *rpoD* gene (Fig. 4C). These results together indicate that the lack of *rpoF* mRNA in D- and E-type bacteria is due to a defect in FlhDC production, but it remains unsolved why *flhD* mRNA is not synthesized in these W3110 variants.

Over 3% of the *E. coli* K-12 genome is concerned with the synthesis, assembly, and function of the flagella (23). In addition, the biosynthesis of a flagellum is costly, mainly because of the large number of flagellin subunits needed for assembly of the filament (23). Under the environmental conditions favorable for bacterial growth, it is reasonable that costly processes such as flagellar biosynthesis, motility, and chemotaxis would be shut off at the level of the master operon. Thus, the lack of σ^{28} may not be disadvantageous in laboratory culture conditions, and might even have selective advantage.

Genetic variations among *E. coli* stock strains. Until 20 years ago, bacterial strains were stored as stab cultures for long periods. However, stab cultures do not guarantee genetic stability. In fact, under the steady state of stationary phase, transposition of mobile genetic elements, which does not depend on DNA replication, contributes to a high degree of genetic instability (28). Recent quantitative analyses based on restriction fragment length polymorphism indicate that a high degree of genetic polymorphism accumulates between subclones isolated from an old stab culture of the strain W3110 and that mobile genetic elements make a major contribution to spontaneous mutagenesis during bacterial storage (26, 27).

Even though our knowledge of the bacterial physiology in stab cultures is poor, it is considered that life in stabs represents particular conditions of stress. Accordingly, stationary-phase *E. coli* cells develop a marked resistance against various stresses such as exposure to heat shock (>50°C), hydrogen peroxide (13), or high salt concentrations (12). Thus, the lack of σ^{38} and/or σ^{28} may be quite serious for survival in stab

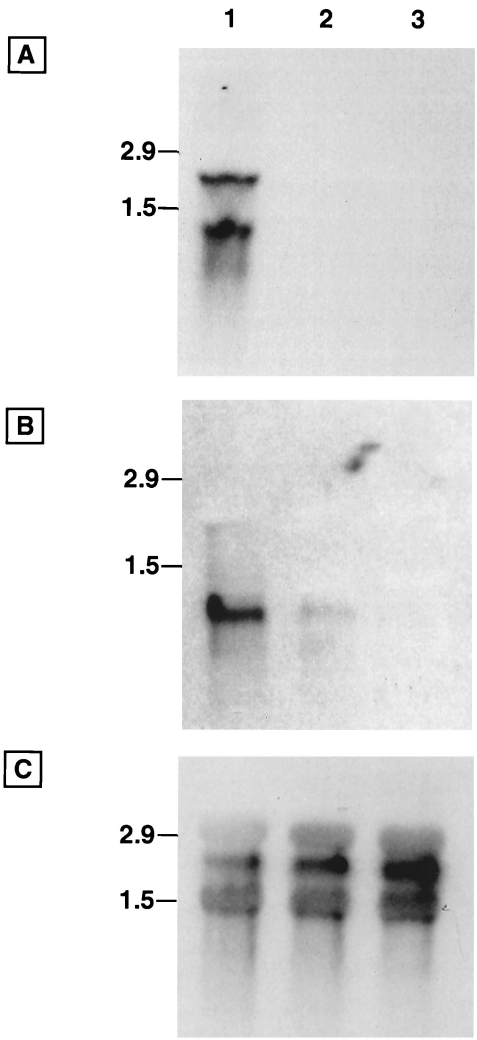

FIG. 4. Northern blot analysis of *rpoF*, *flhDC*, and *rpoD* transcripts. Total RNAs from A-, D-, and E-type W3110 were analyzed by Northern blotting by using probes for the *rpoF* (A), *flhDC* (B), and *rpoD* (C) genes. For Northern blot analysis of *rpoF* RNA, a probe was PCR amplified by using the pair of primers FA1 (with the sequence 5′-GCCGGATCCCAGGCCTACAAGTTGAATTGC) and FA2 (with the sequence 5′-CCCAAGCTTCTGACGTTATAACTTACCC G). PCR products were digested with *Eco*RI to obtain a 722-bp fragment. For Northern blot analysis of the *flhD* transcript, a probe was PCR amplified by using the pair of primers FD1 (with the sequence 5′-GCCGGATCCGATCTGTCAT CACGAATTATTG), and FD2 (with the sequence 5′-CCCAAGCTTAAACAG CCTGTACTCTCTGTTC). PCR products were treated with *Eco*RI to obtain a 1,205-bp fragment. Lane 1, A type; lane 2, D type; lane 3, E type. Migration positions of ribosomal RNAs (1.5 and 2.9 kb) are shown on the left.

cultures. Accordingly, it is likely that these stock lineages have acquired additional mutations which can suppress the σ mutations, even though a majority of those mutations may be indistinguishable by their phenotypes when grown under laboratory culture conditions. However, we cannot exclude that, once into the stationary phase in a stab, motility might not matter or might not be able to be expressed, and therefore the lack of σ^{28} might not be so disadvantageous. Likewise, the lack of σ^{38} might have some unexpected advantages.

Most of the genetic, biochemical, and physiological studies of *E. coli* have so far been carried out using exponentially growing cultures. Nonsporulating bacteria such as *E. coli* in the resting state have long been neglected in genetic, biochemical, and physiological studies, even though starvation is the most frequent state in nature. Therefore, the lack of stress-response σ factors had not been detected. Taking the previously reported (26, 27) and the present findings into account, we propose that for experimental research on *E. coli*, special care should be taken to use a common bacterial stock of strain W3110 such as the glycerol stock maintained in the Bachmann collection, which contains all four σ subunits in intact form.

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