1996

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JOURNAL OF BACTERIOLOGY, Nov. 1996, p. 6443–6450 Vol. 178, No. 22
0021-9193/96/$04.00+0

Role of the *Escherichia coli* FadR Regulator in Stasis Survival and Growth Phase-Dependent Expression of the *uspA*, *fad*, and *fab* Genes

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Received 13 May 1996/Accepted 30 August 1996

The increased expression of the *uspA* gene of *Escherichia coli* is an essential part of the cell's response to growth arrest. We demonstrate that stationary-phase activation of the *uspA* promoter is in part dependent on growth phase-dependent inactivation or repression of the FadR regulator. Transcription of *uspA* is derepressed during exponential growth in *fadR* null mutants or by including the fatty acid oleate in the growth medium of FadR$^+$ cells. The results of DNA footprinting analysis show that FadR binds downstream of the *uspA* promoter in the noncoding region. Thus, *uspA* is a member of the *fadR* regulon. All the *fad-lacZ* fusions examined (*fadBA*, *fadL*, and *fadD*) are increasingly expressed in stationary phase with kinetics similar to that of the increased expression of *uspA*. In contrast, β-galactosidase levels decrease during stationary phase in a *fabA-lacZ* lysogen, consistent with the role of FadR as an activator of *fabA*. The growth phase-dependent increased and decreased transcription of *fad* genes and *fabA*, respectively, is dependent on the status of the *fadR* gene. Cells carrying a mutation in the FadR gene (*fadR*S219N) that makes it nonderepressible exhibit a weak stationary-phase induction of *uspA* and *fad* genes. In addition, cells carrying *fadR*S219N survive long-term stasis poorly, indicating that FadR-dependent alterations in fatty acid metabolism are an integral and important part of the adaptation to stationary phase.

The universal stress protein, UspA, is a member of all starvation and stress stimulons so far studied in *Escherichia coli* except the cold shock response (18, 20). UspA is a cytoplasmic serine/threonine phosphoprotein that is phosphorylated during conditions that induce its synthesis (16). Mutant cells devoid of UspA are impaired in their ability to survive complete and prolonged growth inhibition caused by a variety of starvation and stress conditions, indicating that UspA has a general protective function related to the growth-arrested state (15, 20). In addition, mutations in *uspA* result in an abnormal excretion of acetate during growth of cells on glucose or gluconate, a feature leading to a diauxic growth pattern of *uspA* mutant cultures (19). Moreover, *uspA* mutations affect the timing of protein expression during prolonged periods of growth arrest, accelerating the changes in protein expression that normally occur (20). While the lack of UspA renders the cells less resistant to stasis, constitutively elevated levels of the protein significantly reduce the ability to recover from growth arrest (21).

The *E. coli fadR* gene product functions as a repressor of many unlinked genes and operons encoding proteins involved in long-chain fatty acid transport, activation, and β-oxidation. FadR binds specific operator sites upstream of the fatty acid degradative (*fad*) gene coding sequences to turn off transcription (5). However, FadR can also act as an activator of at least the *fabA* and *fabB* genes which are required for fatty acid biosynthesis (2, 4, 13). Derepression of *fad* genes occurs upon growth of *E. coli* in medium containing long-chain fatty acids (C_{14} to C_{18}). Such growth conditions result in a decrease in the expression of fatty acid biosynthetic (*fab*) genes (2). Long-chain acyl coenzyme A (acyl-CoA) thioesters, generated from exogenous fatty acids, are the effector molecules that regulate fatty acid metabolism at the level of FadR-dependent transcription by binding directly to FadR (24). The FadR–acyl-CoA thioester complex is unable to bind the operator sequence, resulting in transcription of the *fad* genes and the loss of activation of *fabA* transcription (5–7).

In this study, we demonstrate that stationary-phase induction of *uspA* is dependent on the status of the *fadR* gene and that the FadR regulon and *uspA* are induced concomitantly in stationary phase. Moreover, cells that carry a dominant nonderepressible *fadR* allele are impaired in their ability to survive long-term stasis. We discuss the possible physiological assignment of the regulon during growth arrest.

MATERIALS AND METHODS

Bacterial strains and growth conditions. The *E. coli* strains used in this work are listed in Table 1. Cultures were grown aerobically in liquid Luria-Bertani (LB) or M9 (26) medium in Erlenmeyer flasks in a rotary shaker. When required, the media were supplemented with oleate (0.5 mM), glucose (0.4%), glycerol (0.4%), acetate (0.4%), or L-serine (0.4%), and thiamine (10 mM). When appropriate, the media were supplemented with kanamycin (50 μg/ml), carbenicillin (50 μg/ml), tetracycline (20 μg/ml), chloramphenicol (30 μg/ml), and/or streptomycin (200 μg/ml).

General methods. Plasmid DNA was purified by using Qiagen columns (Qiagen, Inc.) according to the protocol provided by the manufacturer. P1 transductions and plasmid transformations were performed as described previously by Miller (14) and Sambrook et al. (26). Culture samples were processed to produce extracts for resolution on two-dimensional polyacrylamide gels by the method of O'Farrell (22) with modifications (28).

Construction of λ-*uspA-lacZ* lysogens and *uspA*::*lacZ*-Km^r insertional mutants. A *uspA*::*lacZ*-Km^r fusion was constructed by inserting the *Sal*I fragment of plasmid pKOK5 (10) containing *lacZ*-Km^r into the *Sal*I site of *uspA* carried on plasmid pTN6093 (19) (Fig. 1A). A fragment containing the *uspA*::*lacZ*-Km^r fusion was integrated into the chromosome of the *E. coli recD* K4633 by linear

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TABLE 1. Bacterial strains and plasmids

Strain or plasmid	Relevant genotype or characteristic	Source or reference
E. coli strains		
MC4100	F⁻ *araD139* Δ(*argF-lac*)*U169 rpsL 150 relA1 flbB5301 deoC1 ptsF25 rbsR*	M. Giskov
TN4100	MC4100 *uspA*::*lacZ*-Km^r	This work
TN4111	TN4100 Δ*cya-851*	This work
TN4112	TN4100 Δ*crp-96 zhd-732*::Tn*10*	This work
TN4131	TN4100 *fadR*::Tn*10*	This work
TN4132	TN4131/pCD126	This work
TN4133	TN4100/pRW22	This work
TN4134	TN4100/pACYC177	This work
TN4135	TN4100 Δ*arcA*::Tn*10*	This work
TN4136	TN4100 Δ*hns-206::bla*	This work
TN4137	TN4100 *dam-13*::Tn*9*	This work
TN4138	TN4100 *fis-767*	This work
TN4139	TN4100 *soxS*::Tn*10*	This work
TN4140	TN4100 *fur*::Tn*10*	This work
TN4141	TN4131/F′ *proAB lacI*^{q} Δ(*lacZ*)M15	This work
TN4142	TN4141/pTN223	This work
AF634	MC4100 λΦ(*uspA-lacZ*)	This work
AF637	AF634 *himA*::*kan*	This work
AF638	AF634 *relA251 spoT207*	This work
AF639	AF634 *relA251*	This work
AF640	AF634 *fadR*::Tn*10*	This work
AF641	AF634 *uspA*::*kan*	This work
W3110	In(*rrnD-rrnE*)*1*	F. C. Neidhardt
ZK126	W3110 Δ*lac*	R. Kolter
TN31	ZK126 *uspA*::*lacZ*-Km^r	This work
LS1345	MC4100 λΦ(*fadB-lacZ*)	DiRusso et al. (6)
LS1346	LS1345 *fadR*::Tn*10*	DiRusso et al. (6)
LS1349	MC4100 λΦ(*fadL-lacZ*)	DiRusso et al. (6)
LS1350	LS1349 *fadR*::Tn*10*	DiRusso et al. (6)
PN428	C600 λΦ(*fadD-lacZ*)	Black et al. (3)
PN424	PN428 *fadR*::Tn*10*	Black et al. (3)
LS1347	MC4100 λΦ(*fabA-lacZ*)	DiRusso et al. (6)
LS1348	LS1347 *fadR*::Tn*10*	DiRusso et al. (6)
RW11	*fadR*::Tn*10*	C. C. DiRusso
K4633	*recD*	D. Friedman
K5302	*himA*::*kan*	D. Friedman
RH76	MC4100 Δ*cya-851*	R. Hengge-Aromis
BD4100	MC4100 Δ*crp96 zhd-732*::Tn*10*	R. Hengge-Aromis
MG1655	Wild type	Laboratory stock
LN1655	MG1655 Δ*fis-767*	L. Nilsson
GM2929	*dam-13*::Tn*9*	M. G. Marinus
TN3151	W3110 *uspA*::*kan*	T. Nyström
PD32	MC4100 Δ*hns-206*::*bla*	V. deLorenzo
CF1948	W3110 Δ*relA251* Δ*spoT207*	M. Cashel
QC1554	*soxS*::Tn*10*	D. Touati
QC2085	Δ*arcA*::Tn*10*	D. Touati
Plasmids		
pTN6091	5-kb *Pst*I fragment harboring *uspA*	T. Nyström
pTN6093	2.3-kb *Kpn*I-*Pst*I fragment harboring *uspA*	T. Nyström
pTN6095	*Xho*I-*Pst*I fragment harboring *uspA*	T. Nyström
pKOK5	*lacZ*-Km^r operon fusion vector	Kokotek and Lotz (10)
pTL61T	*lacZ* operon fusion vector	Linn and St. Pierre (11)
pCD126	Wild-type *fadR* on pACYC177	C. C. DiRusso
pRW22	*fadR*S219N on pACYC177	C. C. DiRusso
pTN223	P_{tac}-*uspA* operon fusion	Nyström and Neidhardt (21)
pAF602	1.2-kb *Sac*II-*Pst*I fragment harboring *uspA*	A. Farewell

transformation and subsequently moved by P1 transduction into strain MC4100 (Fig. 1A). The lack of UspA synthesis in these strains was confirmed by two-dimensional gel electrophoretic analysis.

A plasmid-borne P_{uspA}-*lacZ* fusion was constructed by inserting the *Sma*I-*Sal*I fragment of pTN6095 (*Xho*I-*uspA*-*Pst*I [18]), containing the *uspA* promoter, into plasmid pTL61T (11) (Fig. 1B) digested with the same enzymes. The *uspA-lacZ* fusion was recombined into the chromosome by the method of Simons et al. (27) using λ phage RS45 (Fig. 1B). One strain, AF634, monolysogenic for λ phage carrying P_{uspA}-*lacZ* was isolated for further studies.

Measurements of β-galactosidase activity. The β-galactosidase levels were measured as previously described by Miller (14) with modifications (1). Samples were measured spectrophotometrically at 420 nm (β-galactosidase) and 550 nm to correct for remaining cell debris. The β-galactosidase activity is expressed as follows: $[OD_{420} - (1.75 \times OD_{550})]/(OD_{600}$ culture $\times$ reaction time $\times$ volume), where OD_{420} is the optical density at 420 nm.

FadR-*uspA* footprinting with DNase I. A 377-bp fragment including positions −214 to +163 of *uspA* was amplified from plasmid pAF602 using the PCR and oligonucleotides 5′ GTCAGAATTCTCCCGATACGCTGCCAATCAGTT and 5′ TCGAGGATCCAGGTCGACCGCGATGAGAATGTGT as primers. The underlined nucleotides include those added to generate *Eco*RI and *Bam*HI restriction sites, respectively. The amplified fragment was cloned into pUC19 to generate pMD107-19. The insert was verified by DNA sequencing. For DNase I footprinting of the template strand, the reverse primer was phosphorylated with [γ-^{32}P]ATP (7,000 Ci/mmol) and polynucleotide kinase. The PCR primers used for the coding strand were 5′ TAAGCAAGGCGGATTGA and 5′ CCGCGAT GAGAATGTGTT. Thirty-five cycles of PCR were performed; 1 PCR cycle consists of 1 min at 94°C, 1 min at 36°C, and 1 min at 72°C. The fragment of interest was gel purified. DNA was treated with FadR and DNase I as described previously by DiRusso et al. (5).

RESULTS

The *uspA* gene is transcriptionally activated during entry of cells into stationary phase. β-Galactosidase activities were determined for *E. coli* strains lysogenic for λ phage carrying P_{uspA}-*lacZ* fusions (strain AF634) and a strain (TN4100) in which *uspA*::*lacZ* was integrated by double homologous recombination into the normal *uspA* location on the chromosome. The *uspA* promoter was induced in early stationary phase, regardless of whether the fusion was located at the λ*att* site or the normal *uspA* site on the chromosome (Fig. 2A). The same results were obtained when the *uspA*::*lacZ* fusion was inserted into the chromosome of *E. coli* W3110Δ*lac* instead of MC4100 (not shown). The induction was independent of functional UspA, since strain AF634, made *uspA*::*kan* (AF641), exhibited β-galactosidase levels during exponential growth and in stationary phase identical to those of the parent (Fig. 2A). The induction upon entry into stationary phase of the *uspA-lac* fusions was approximately fourfold. This is lower than the induction ratio previously reported when the UspA protein was examined (18, 20), indicating that perhaps there is post-transcriptional as well as transcriptional regulation of *uspA*. The transcriptional levels of *uspA-lacZ* were independent of growth rate (Fig. 2B), as are the levels of UspA synthesis (18).

Induction of *uspA* is dependent on the status of the *fadR* gene. To assess whether known regulatory genes control P_{uspA} activity, mutations in such genes were transduced to strains AF634 and TN4100 and their effects on β-galactosidase activity were determined. Regulators tested included *cya*, *crp*, *soxS*, *rpoH*, *arcA*, *fur*, *relA*, *fis*, *dam*, *himA*, *oxyR*, *rpoS*, and *hns* (Fig. 3). No significant effects on growth phase-dependent *uspA-lacZ* expression were found except for the *fadR* mutation. The *crp* and the *cya* mutations significantly increased expression from P_{uspA} both during exponential growth and in stationary phase (Fig. 3). The activity of the *uspA* promoter was reduced in cells carrying a *himA* insertion mutation, but like mutations in *crp* and *cya*, the effects were seen in both logarithmic growth and stationary phase, and the ratio of β-galactosidase levels in stationary phase to that in exponential growth was very similar to this ratio in the wild-type strain. In other words, wild-type as well as *cya*, *crp*, and *himA* strains had a four- to fivefold induction of *uspA-lac* in stationary phase over that of log phase.

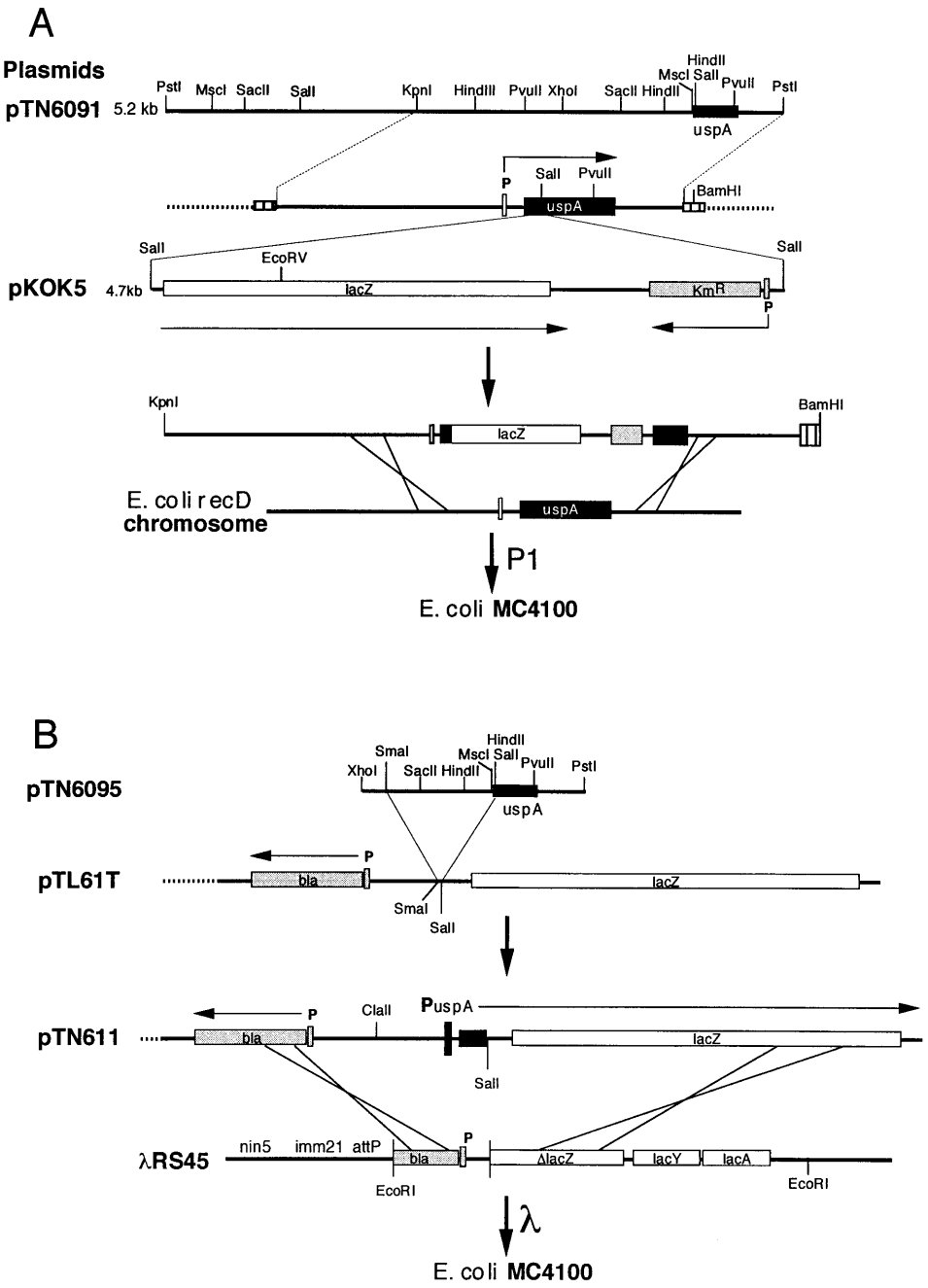

FIG. 1. Schematic drawing of the construction of P_{uspA}-*lacZ* fusions integrated at the normal *uspA* location on the chromosome (A) or at the λ*att* site (B). The figure is not drawn to scale. (A) The *uspA*::*lacZ*-Km^r fusion was constructed by inserting the *Sal*I fragment of plasmid pKOK5 (10) containing *lacZ*-Km^r into the *Sal*I site of *uspA* carried on plasmid pTN6093 (19). The correct orientation of *lacZ* with respect to the *uspA* promoter was confirmed by digesting the resulting plasmid (pTN6099) by *Bam*HI and *Eco*RV. This plasmid was linearized by *Kpn*I-*Bam*HI digestion, and the *uspA*::*lacZ*-Km^r fusion was integrated into the chromosome of the *E. coli recD* K4633 by linear transformation and subsequently moved by P1 transduction into strain MC4100. (B) A plasmid-borne P_{uspA}-*lacZ* fusion was constructed by inserting the *Sma*I-*Sal*I fragment of pTN6095, containing the *uspA* promoter, into plasmid pTL61T (11) digested with the same enzymes. In the resulting plasmid (pAF632), *lacZ* is transcribed from the *uspA* promoter. λ phage RS45 (27) contains the 3′ end of the *lacZ* gene and the 5′ end of *bla*; the gene fusion was transferred to λRS45 by homologous recombination between the plasmid and phage. λRS45 was grown on MC4100 carrying the new plasmid, and recombinant phages (expressing LacZ) were identified by plating the lysate on MC4100 in medium containing X-Gal (5-bromo-4-chloro-3-indolyl-β-D-glucuronic acid).

In contrast, mutations in *fadR* significantly affected growth phase-dependent expression from the *uspA* promoter. The *fadR* null mutant exhibited significantly higher levels of β-galactosidase in the *uspA-lacZ* fusion during exponential growth over that of the isogenic parent, and there was only a small degree of induction upon entry into stationary phase (1.6-fold) (Fig. 4A). This phenotype was complemented with plasmid pCD126 carrying the wild-type *fadR* allele (Fig. 4A). Levels of β-galactosidase were also significantly higher during exponential growth in the FadR$^+$ strain when the long-chain fatty acid oleate was added to the medium (Fig. 4B). No effect was observed with the addition of glucose or glycerol (Fig. 4B). In

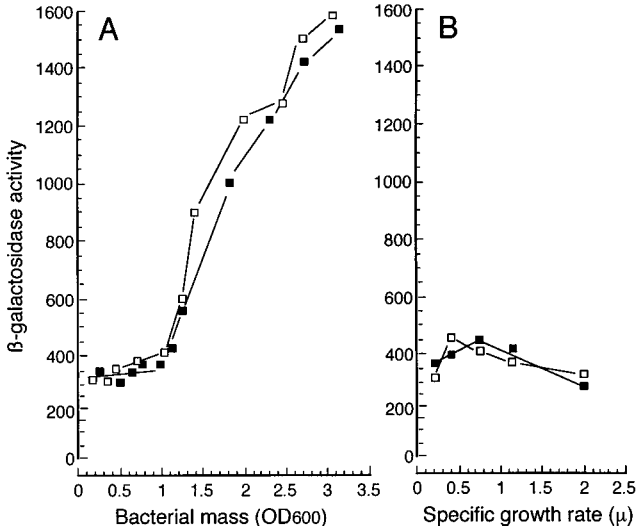

FIG. 2. (A) Expression of the *uspA* promoter in λ*uspA-lacZ* lysogens AF634 (wild type) and AF641 (*uspA*::*kan*) during exponential growth and in stationary phase. β-Galactosidase activity is plotted as a function of cell mass (optical density at 600 nm [OD_{600}]). Cell growth typically started to cease at an OD_{600} of 1.0 in the growth conditions examined (LB medium with glucose, 37°C, aerobic conditions). (B) Expression of the *uspA* promoter during exponential growth of strains AF634 and AF641 in minimal medium supplemented with glucose plus amino acids, glucose, glycerol, succinate, or L-serine. β-Galactosidase levels are plotted as a function of growth rate which is expressed as μ, the first-order growth rate constant. Symbols: □, AF634; ■, AF641.

addition, cells carrying a transdominant nonderepressible mutation in *fadR* (change of Ser-219 to Asn [*fadR*S219N] [24]) on plasmid pACYC177 exhibited weaker induction of *uspA* than did cells carrying the wild-type allele or vector alone (Fig. 4C). Taken together, these observations suggest that the increased expression of *uspA* is in part an effect of FadR-dependent derepression during entry of cells into stationary phase. However, regulatory factors other than FadR are likely to be involved in the induction of *uspA*, since a *fadR* null mutant still exhibits some induction of *uspA-lacZ* upon entry into stationary phase.

The effect of *fadR* mutations on *uspA* expression suggests that UspA may have a role in fatty acid metabolism. A *uspA* null mutant grows on long-chain fatty acids, such as oleate, as the sole source of carbon, albeit with a somewhat slower growth rate than the isogenic wild-type MC4100 (Fig. 5). Therefore, *uspA* does not appear essential for fatty acid degradation, but it may be involved in fatty acid biosynthesis and/or membrane composition and integrity as discussed below.

FadR protein binds upstream of the *uspA* coding region. To test if the effect of FadR on uspA expression was direct, we analyzed the binding of FadR to *uspA* by DNase I footprinting. As shown in Fig. 6, FadR clearly binds upstream of the *uspA* coding region at a position +88 to +104 relative to the start of transcription. Examination of the sequence shows that the region where FadR binds has sequence similarity to other FadR binding sites (Table 2). Thus, *uspA* should be considered part of the *fadR* regulon. A second site (positions −4 to +13) also shows some weak FadR binding (Fig. 6), but this site exhibits little homology to the other FadR binding sites.

The *fadR* regulon is derepressed in stationary phase. The results in the preceding sections indicate that the FadR repressor is inactivated or is itself repressed during entry of cells into stationary phase. If so, the *fad* genes and operons, derepressed by fatty acids and repressed by FadR, should also be increasingly expressed in stationary phase while the expression of the fatty acid biosynthetic genes activated by FadR should be reduced. We examined β-galactosidase levels in three strains lysogenic for λ phage carrying *lacZ* fusions to the *fadBA*, *fadD*, and *fadL* promoters. The *fadBA* operon encodes two proteins of the β-oxidation multienzyme complex; *fadD* encodes acyl-CoA synthetase, and *fadL* encodes the outer membrane fatty acid transport protein (2). We found that all *fad* fusions examined were increasingly expressed in stationary phase with kinetics similar to that of expression of *uspA* (Fig. 7). Comparison of growth phase-dependent expression in the wild-type and *fadR* mutant demonstrated that the increased expression was at least partly dependent on the *fadR* gene (Table 3). In the medium used, *fadBA* and *fadD* expression was also dependent on *cya* and *crp* (not shown), while no effects of mutations in these genes could be observed in the *fadL-lacZ* fusion. These results are consistent with previous work showing that *fadB* and *fadD* are regulated by cyclic AMP receptor protein while *fadL* is not (3, 6).

We also examined β-galactosidase levels in a strain lysogenic for λ phage carrying a *lacZ* fusion to the *fabA* gene encoding β-hydroxydecanoyl-acyl carrier protein dehydratase which is required for unsaturated fatty acid biosynthesis (2). β-Galactosidase levels decreased in the *fabA-lacZ* lysogen in stationary phase (Fig. 7), consistent with inactivation of FadR. *fabA* expression was much lower during logarithmic growth in the *fadR* background and was not further regulated by growth phase transition (Table 3). As expected, no effects on *fabA* expression were observed by mutations in either *cya* or *crp* (not shown) (6).

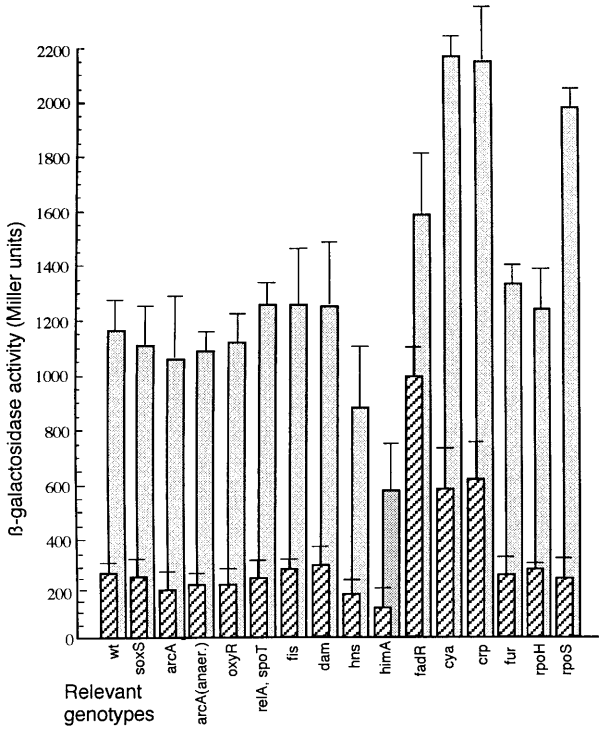

FIG. 3. Effects of a number of regulatory mutations on the expression of the *uspA* promoter. β-Galactosidase levels were determined during logrithmic growth (hatched bars) and approximately 1 h after growth ceased (gray bars). Cells were grown in LB medium with glucose. The strains used are described in Table 1. wt, wild type (MC4100); anaer., anaerobic.

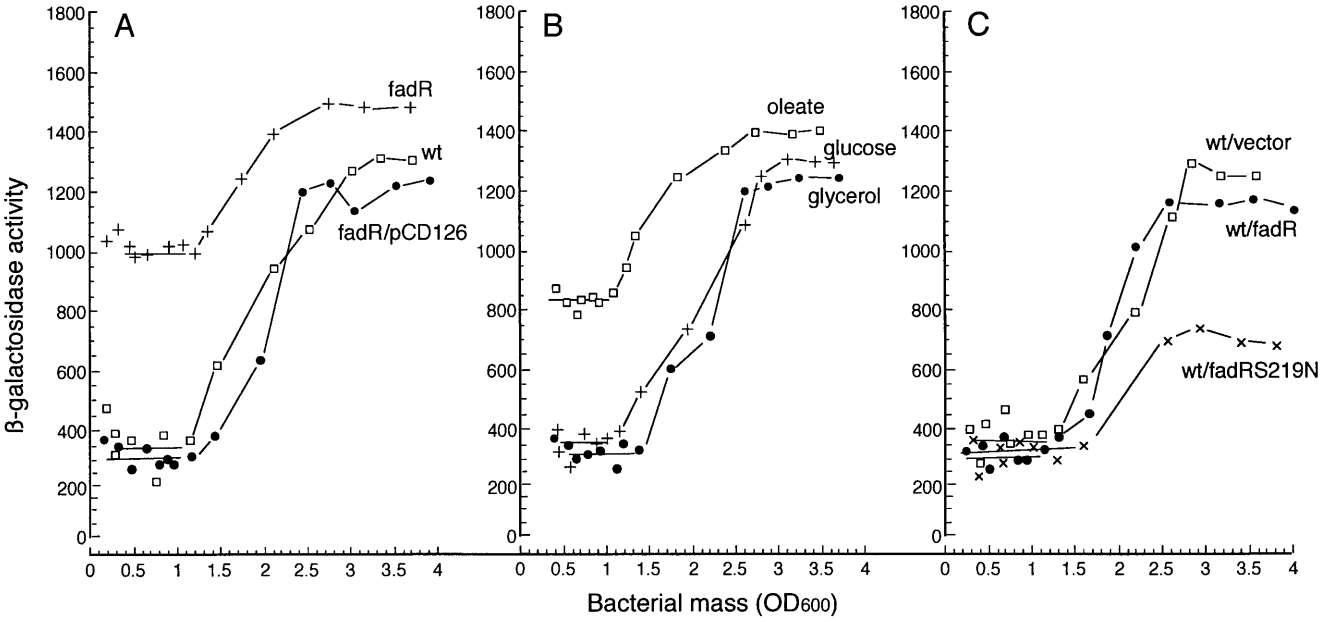

FIG. 4. (A) Effects of a *fadR* mutation on the levels of expression of the *uspA* promoter. β-Galactosidase levels were determined for strain TN4100 (wild type [wt]), TN4131 (*fadR*), and TN4131 carrying the wild-type *fadR* allele on plasmid pCD126 (fadR/pCD126). Cells were grown in LB medium with glucose. (B) Effect of including the fatty acid oleate in the growth medium on the levels of expression of the *uspA* promoter. The wild-type strain TN4100 was grown in LB medium supplemented with either oleate, glucose, or glycerol. (C) Effects of the transdominant nonderepressible mutation *fadR*S219N on the levels of expression of the *uspA* promoter. β-Galactosidase levels were determined for strain TN4100 carrying the nonderepressible *fadR*S219N allele (wt/fadRS219N), the wild-type allele (wt/fadR) or vector alone (wt/vector). Cells were grown in LB medium with glucose. The same results were obtained in a *fadR* null mutant carrying the *fadR*S219N plasmid as were obtained with a *fadR*$^+$ cell (data not shown).

Derepression of the *fadR* regulon is important for stasis survival. To examine whether a failure to derepress the *fadR* regulon affects the cell's ability to survive stasis, we compared stationary-phase survival of cells carrying either the nonderepressible *fadR*S219N allele, the wild-type allele, or a vector plasmid. As depicted in Fig. 8, cells carrying *fadR*S219N were more sensitive to long-term stasis than cells carrying vector or the wild-type *fadR* allele. The same results were obtained whether the strains carrying the plasmids were *fadR*$^+$ or *fadR* (data not shown). Because it is known that *uspA* mutants survive poorly in stationary phase (20), we examined whether the poor ability of *fadR*S219N cells to survive was the result of their reduced ability to increase *uspA* expression during entry into stationary phase (Fig. 4C). We approached this question by transforming the cells carrying the *fadR*S219N plasmid with the compatible plasmid pTN223, which is pBR322 carrying an operon fusion between the *tac* promoter and *uspA* (21). Cells were starved in the presence of 50 μM IPTG (isopropyl-β-D-thiogalactopyranoside) which induces UspA synthesis eightfold (21), an induction level comparable to that reached in wild-type cells subjected to different starvation and stress conditions. However, *uspA* induction could not alleviate the harmful effect of the *fadR*S219N mutation (Fig. 8).

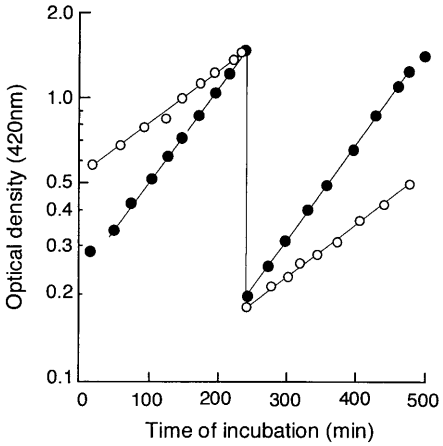

FIG. 5. Effect of *uspA* mutation on the growth of *E. coli* on the fatty acid oleate. The wild-type strain MC4100 (●) and its isogenic *uspA*::*kan* derivative (○) were grown aerobically in oleate (0.5 mM) minimal M9 medium at 37°C. The optical density at 420 nm is plotted on a logarithmic scale as a function of time. The vertical line indicates dilution of the culture.

DISCUSSION

We report here that the expression of *uspA* is dependent on the status of the *fadR* gene. A *fadR* null mutant exhibits derepressed expression levels of *uspA* during exponential growth, while cells carrying a transdominant nonderepressible *fadR* allele increase *uspA* expression poorly during entry into stationary phase. The effects of *fadR* mutations on *uspA* expression suggest that UspA may have a role in fatty acid metabolism; all genes demonstrated to be regulated by FadR so far have assignments in fatty acid uptake, activation, or metabolism. However, a *uspA* null mutant grows on long-chain fatty acids, such as oleate, as the sole source of carbon, albeit with a somewhat slower growth rate than that of the isogenic wild type. Therefore, *uspA* does not appear essential for fatty acid degradation. Alternatively, UspA may be involved in fatty acid or membrane lipid biosynthesis. If so, the fact that FadR appears to repress *uspA* expression suggests that UspA may function as a governor that either reduces the pace of biosynthesis or affects the overall composition of membrane fatty acids. Several observations indicate that UspA may be involved in

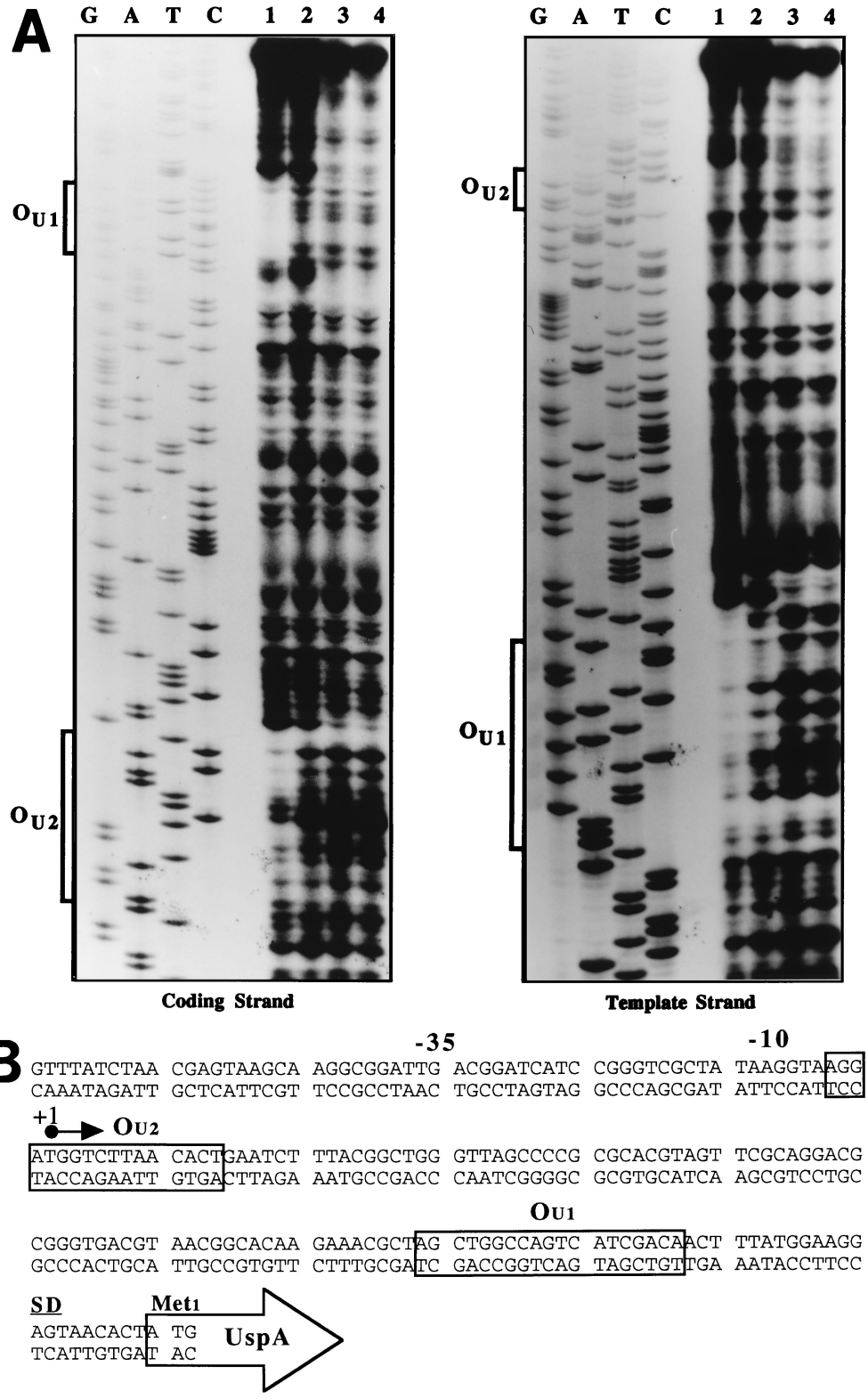

FIG. 6. (A) Identification of the FadR binding site within *uspA* and the sequence of the *uspA* promoter region. The products of sequencing reaction using the universal M13 reverse primer and pMC107-19 as a template are identified as G, A, T, and C. Lanes 1, 2, and 3 contain 4 mM, 400 nM, and 40 nM FadR, respectively. Lanes 4 have no added FadR. (B) Sequence of the *uspA* promoter region. The −35 and −10 regions of the *uspA* promoter, the transcription start site (small arrow), the FadR binding sites (O_{U1} and O_{U2}), the Shine-Dalgarno sequence (SD), and the first codon of *uspA* (large arrow) are indicated.

TABLE 2. Identified and predicted FadR binding sites

Gene	Sequence[a]		No. of bases in common with[b]:	
			O_B	O_A
fadB	ATCTGGTACGACCAGAT	+17	(17)	10
fadL1	AGCTGGTCCGACCTATA	−9	11	11
fadL2	CACTGGTCTGATTTCTA	+16	7	11
fadD1	AGCTGGTATGATGAGTT	−29	12	9
fadD2	GGCTGGTCCGCTGTTTC	−115	7	8
fadE	AAGTGGTCAGACCTCCT		10	11
fabA1	AACTGATCGGACTTGTT	−31	10	(17)
fabB	GGCTGATCGGACTTGTT	−31	9	15
uspA	AGCTGGCCAGTCATCGA	+104	8	9
Consensus sequence	ANCTGGTCNGANC$^{T}_{A}$GTN			

[a] Sequence data were taken from Black and DiRusso (2) or unpublished results (4). The position relative to the start of transcription as determined by primer extension analysis is shown. The *fadE* transcriptional start site has not been mapped.

[b] Number of bases in common with the binding sites of *fadB* (O_B) or *fabA1* (O_A).

membrane integrity. We have noted that *uspA* mutants are extremely sensitive to weak acids and exhibit an altered pH range for growth. Also, *uspA* mutants exhibit a diauxic type of growth when grown on glucose or gluconate. This phenotype was found to be the result of significant amounts of acetate being excreted into the medium of the mutant strain which, after an extended lag, was able to grow on this acetate (19). The excretion of acetate may be the result of an increased leakiness of the membrane of the mutant. In addition, it has been observed that UspA copurifies with the β-ketoacyl-acyl carrier protein synthase I (product of *fabB*) (23). These observations have provided the impetus to further explore possible alterations in membrane lipid composition due to the lack or overproduction of UspA.

The degradation of membrane constituents is an integral part of the dwarfing process of nondifferentiating bacteria subjected to starvation for exogenous carbon and energy. This process generates small, coccoid cells (e.g., see references 17 and 25), and it includes extensive degradation of endogenous membrane phospholipids (9, 12), an activity proposed to provide the cell with carbon and energy for maintenance requirements. The derepression of the *fadR* regulon (either by inactivation or repression of FadR) during entry of cells into stationary phase suggests that this regulon, apart from being required for growth on exogenous long-chain fatty acids, may be involved in providing the growth-arrested cell with endogenous carbon and energy during dwarfing. The derepression of the regulon would make physiological sense in that fatty acids generated from degradation of membrane lipids during dwarfing could be scavenged by the activity of the acyl-CoA synthetase (product of the *fadD* gene) to generate acyl-CoA. Acyl-CoA is further catabolized by the β-oxidation enzymes (encoded by *fadBA*, *fadE*, *fadFG*, and *fadH*) to generate acetyl-CoA, a source of carbon and energy. Indeed, cells carrying the nonderepressible *fadR*S219N allele were more sensitive to long-term stasis than the isogenic parent (Fig. 8A). This mutated form of FadR has lost its ability to bind the effector molecule acyl-CoA but remains capable of binding DNA, repressing *fad* genes, and activating *fab* genes (24). Starvation also caused significant lysis of *fadR*S219N cells (Fig. 8B) which may be due in part to an altered membrane lipid composition.

The *fadR* regulon may be involved in the alterations in membrane phospholipid composition that take place during growth arrest. Specifically, *fadR* null mutants are known to synthesize more cardiolipin (diphosphatidylglycerol) and less phosphatidylglycerol (29). Thus, derepression of the *fadR* regulon during stationary phase would presumably result in increased levels of membrane cardiolipin; a feature typical of stationary-phase cells of *E. coli* (8). It may be argued that the

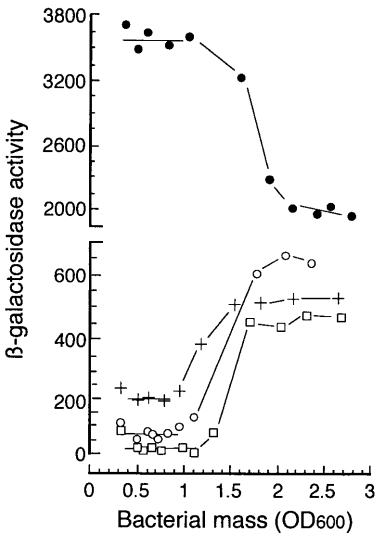

FIG. 7. Levels of β-galactosidase in λ lysogens carrying *fadB-lacZ* (□), *fadD-lacZ* (○), *fadL-lacZ* (+), and *fabA-lacZ* (●) during growth and in stationary phase. Consult the legend to Fig. 2 for details.

TABLE 3. Transcription levels of FadR-dependent genes during exponential growth and in stationary phase

Strain	Relevant genotype	Growth phase	β-Galactosidase activity[a]	Induction ratio[b]
LS1345	λΦ(*fadB-lacZ*)	Log	35 (11)	
		Stationary	455 (21)	13.0
LS1346	*fadR* λΦ(*fadB-lacZ*)	Log	467 (56)	
		Stationary	1,077 (81)	2.3
LS1349	λΦ(*fadL-lacZ*)	Log	206 (32)	
		Stationary	505 (23)	2.5
LS1350	*fadR* λΦ(*fadL-lacZ*)	Log	493 (21)	
		Stationary	827 (73)	1.7
PN428	λΦ(*fadD-lacZ*)	Log	61 (8)	
		Stationary	651 (32)	10.7
PN424	*fadR* λΦ(*fadD-lacZ*)	Log	341 (115)	
		Stationary	1,007 (56)	3.0
LS1347	λΦ(*fabA-lacZ*)	Log	3,680 (131)	
		Stationary	1,140 (103)	0.31
LS1348	*fadR* λΦ(*fabA-lacZ*)	Log	580 (40)	
		Stationary	500 (35)	0.86

[a] Values are expressed as described in Materials and Methods under "Measurements of β-galactosidase activity". Numbers in parentheses are standard deviations.

[b] Induction ratios are expressed as the β-galactosidase activity levels obtained in stationary phase divided by β-galactosidase activity levels for cells in the exponential phase of growth.

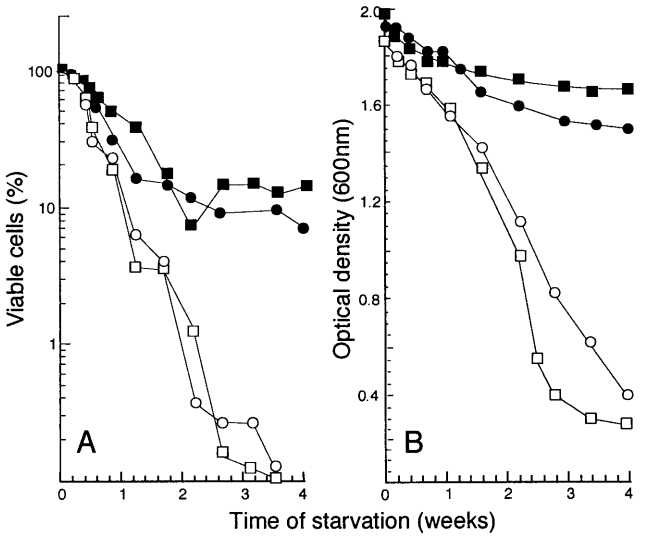

FIG. 8. Survival of cells carrying the vector (●), the transdominant nonderepressible mutation *fadR*S219N (□), the wild-type *fadR* allele (■), or *fadR*S219N together with pTN223 (○). Strains were grown aerobically in LB medium at 37°C. After growth ceased, incubation was monitored for 4 weeks under the same conditions. (A) Viable cells. Viable cells were counted as colonies plated on LB medium after the appropriate dilution. (B) Optical density at 600 nm was read. One hundred percent viability corresponds to the number of viable cells counted 5 h after growth (measured by optical density) was arrested. At this time there was no further increase in cell number due to reductive division.

*fadR*S219N mutant would contain less cardiolipin in stationary phase because of its inability to derepress the *fadR* regulon. Cardiolipin is one of the major phospholipids in *E. coli*, but its essentiality and specific functions are unclear. It is mainly synthesized by cardiolipin synthase, encoded by *cls*, whose activity increases about 10-fold in stationary phase, while the activities and levels of other enzymes of phospholipid biosynthesis decrease (8). A null *cls* mutant is able to grow but survives stationary phase poorly (8). Thus, it is possible that the sensitivity of *fadR*S219N mutants to stasis is related to cardiolipin metabolism rather than a reduced ability to utilize endogenous fatty acids for maintenance requirements. However, the role of FadR in *cls* transcription or cardiolipin synthase activity remains to be elucidated. Future work will also include examination of the importance of alterations in membrane composition under the diverse stresses which are known to induce UspA and whether FadR regulation of UspA is sufficient to induce the protein in all these conditions. The mechanism by which FadR-dependent regulation in stationary phase occurs will also be a subject of future study; preliminary work indicates that FadR may not be responding to acyl-CoA thioesters synthesized by *fadD*, thus either FadR is itself repressed or is responding to a signal molecule synthesized by a novel *fadD*-independent mechanism in stationary phase. The role of UspA in growth phase alterations of membrane composition also remains to be elucidated.

ACKNOWLEDGMENTS

Strains were generously provided by David Friedman, Victor de-Lorenzo, Mike Cashel, Danièle Touati, Regine Hengge-Aronis, F. Neidhardt, R. Kolter, M. Giskov, M. Marinus, H. Nash, E. Granston, Jr., and Lars Nilsson.

This work was supported by grants from the Swedish Natural Science Research Council (NFR) to T.N. and National Science Foundation grant MCB-9407220 to C.D.R.

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