Proc. Natl. Acad. Sci. USA
Vol. 90, pp. 11004–11008, December 1993
Biochemistry

spoT-dependent accumulation of guanosine tetraphosphate in response to fatty acid starvation in *Escherichia coli*

(accD mutation/cerulenin/phospholipids)

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Contributed by Masayasu Nomura, August 13, 1993

ABSTRACT **We previously isolated a mutant of *Escherichia coli* that is preferentially affected in the synthesis of rRNA and has a mutation in the gene (*accD*) encoding a subunit of acetyl-CoA carboxylase. Using this mutant and other mutants of the pathway for fatty acid and phospholipid biosynthesis as well as cerulenin, a specific inhibitor of fatty acid synthesis, we show that (*i*) inhibition of fatty acid synthesis in the presence of both a carbon source and all 20 amino acids stimulates the accumulation of guanosine tetraphosphate (ppGpp) and leads to preferential inhibition of rRNA synthesis, (*ii*) this ppGpp accumulation is *spoT* dependent, and (*iii*) the generation of the metabolic signal that stimulates this *spoT*-mediated response probably does not depend on either phospholipid starvation or a significant reduction in the level of ATP.**

It is well known that amino acid starvation in *Escherichia coli* leads to accumulation of guanosine tetraphosphate (ppGpp) and cessation of rRNA synthesis. This phenomenon, which depends on the presence of the *relA* gene product, is called the stringent response. In addition to cessation of rRNA synthesis, many other biochemical alterations take place in a *relA*-dependent manner upon amino acid starvation—e.g., inhibition of protein synthesis and stimulation of the transcription of some amino acid biosynthetic operons (reviewed in ref. 1). These alterations are likely beneficial to starved cells and appear to be mediated by the effector molecule, ppGpp. Besides amino acid starvation, it is also known that carbon starvation or nutritional deprivation leads to inhibition of stable RNA synthesis accompanied by accumulation of ppGpp (reviewed in ref. 1), and the *spoT* gene is required for this response (1, 2). It appears that *E. coli* cells have evolved mechanisms whereby they monitor nutritional states and exert appropriate adaptive response reactions, for which ppGpp plays an important role.

We now report that *E. coli* cells respond to a deficiency in fatty acid supply by forming ppGpp and inhibiting stable RNA synthesis and that this response is, at least in part, *spoT* dependent.

MATERIALS AND METHODS

Strains and Media. All strains used were derivatives of *E. coli* K-12. Strain NO3978 carries a temperature-sensitive (ts) mutation in *accD* and was derived from NO3979 (a *relA1* derivative of strain GM1; see ref. 3). NO3980 is a derivative of NO3940 (4), carries a Tn*10* insertion (*zfb-1*::*Tn10*; ref. 5) and was used in some experiments as a (*accD*$^+$) wild type. MG1655 (wild type) and its derivative CF1693, which is Δ*relA251*::*kan*, Δ*spoT207*::*cat* (2), were obtained from Michael Cashel (National Institutes of Health). A Δ*relA251*::*kan spoT*$^+$ strain (NO3982) was constructed by introducing the *relA* allele from the latter into MG1655 by P1 transduction. CY50 is a *fabA2*(ts) *pyrD*$^+$ transductant derivative of KL185 (*galK35, pyrD34, trp-45, his-68, rpsL118, malT1, xylA7, mtlA2, thi-1*; ref. 6) and was obtained from the *E. coli* Genetic Stock Center (Yale University). NO3983 is a *spoT*$^+$ derivative of BB26-36 [a gift from Robert Bell (Duke University); this strain carries a mutation in *plsB* in addition to being deficient in aerobic glycerol-3-phosphate dehydrogenase (*glpD*), alkaline phosphatase (*phoA*), and constitutive for the transport of glycerol 3-phosphate (*glpT*) and for glycerol kinase (*glpK*); see ref. 7]. Minimal medium was Mops (8) or AB (9), which, when indicated, was supplemented with 0.4% glucose; 20 μg of glycerol per ml; amino acids in quantities according to ref. 8; and 10 μg of thiamine, 40 μg of uridine, and 40 μg of lysine per ml. All experiments were conducted at 37°C unless otherwise stated.

Relative Differential Synthesis Rate of rRNA. Cultures at 0.2–0.4 OD_{600} were labeled for 1 min with [^{3}H]uridine (100 μC/ml; 1 μC = 37 kBq). Cells were lysed in boiling lysis solution containing 2.5% SDS (10). Labeled RNA was isolated and the amount of radioactive rRNA was determined by RNA·DNA hybridization with a ribosomal DNA (rDNA) probe essentially as described (10). The rDNA probe consisted of a 2-kb *Sal* I–*Sma* I fragment encompassing the distal portion of the *rrnA* 16S gene, the spacer, and the proximal half of the 23S *rrnA* gene cloned into the *Sal* I and *Sma* I sites of M13mp9. M13mp9 without insert served as the control probe. The fraction of total radioactive RNA that hybridized to the rDNA probe was then determined for each sample, and the values obtained were normalized to a control sample. This normalization eliminates the necessity for various corrections, such as the size of the rDNA probe, and allows comparison of the differential synthesis rates of rRNA (the ratio of the rRNA synthesis rate to the total RNA synthesis rate or R_r/R_t) among various samples; hence, the normalized values are called the "relative differential synthesis rate of rRNA."

RNA and Protein Accumulation. Cells were continuously labeled with 5 μC of [^{3}H]uridine and 0.25 μC of [^{14}C]lysine per ml with carrier uridine and lysine, each at 40 μg/ml. At various times, 0.1-ml samples of cultures were precipitated with CCl_3COOH, and their radioactivities were determined.

Measurements of ppGpp. Cultures were continuously labeled for at least three generations prior to sampling with 50–100 μC of [^{32}P]orthophosphate per ml in low-phosphate (about 0.2–0.3 mM) medium. Samples (0.1 ml) were mixed with 10 μl of 11 M formic acid on ice, and cell debris and inorganic phosphate were removed as described (11). Equal volumes of these formic acid extracts were chromatographed in one or two dimensions on polyethyleneimine (PEI) cellulose TLC plates (11, 12). For quantification of ppGpp, an area equal in size to each ppGpp spot between ppGpp and GTP

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Abbreviations: ACP, acyl-carrier protein; ts, temperature sensitive.
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was cut out, and the radioactivity in this region was subtracted as background from that in ppGpp spots.

Phospholipid Measurements. To measure accumulation, cells were labeled with ^{32}P as described for ppGpp measurements. ^{32}P-labeled phospholipids were extracted with methanol/chloroform (13) and their amounts were determined. For measurements of synthesis rates, 0.35 ml of culture cells grown in low-phosphate medium was pulse-labeled with [^{32}P]orthophosphate at 100 μC/ml. Samples (0.1 ml) were taken at 0, 2.5, and 5 min. The difference between the amount of [^{32}P]phospholipid at 2.5 min and that at 5 min was used to estimate the synthesis rate.

RESULTS

A Temperature-Sensitive *accD* Mutant Defective in rRNA Synthesis. We previously isolated a ts mutant of *E. coli* that shows a preferential decrease in transcription from rRNA gene promoters at 34°C, a permissive temperature used for mutant isolation (L. Vu, D. Hirota, J.K., and M.N., unpublished data; see ref. 14). The mutation has been mapped to *accD* at 50 min on the *E. coli* chromosome. The *accD* gene encodes a subunit of acetyl-CoA carboxylase (15), which catalyzes the first step in the fatty acid biosynthetic pathway (16) (Fig. 1), and the mutation changes glycine to aspartate at position 214 of the 304-amino acid protein (14).

Table 1 shows the effect of a shift up in temperature on the differential rRNA synthesis rate (R_r/R_t, see *Materials and Methods*) of the mutant compared with its wild-type parent. At the permissive temperature (34°C), the mutant showed a lower R_r/R_t ratio compared with the wild type. Thirty minutes after temperature shift to 42°C, this ratio decreased further to only one-third of the wild type ratio, suggesting a preferential inhibition of rRNA synthesis compared with total RNA synthesis in the mutant. The decrease in R_r/R_t was found to correlate with an increase in the level of ppGpp in the mutant relative to the wild type after the temperature shift to 42°C (Fig. 2). Since the mutant background is *relA1*, the accumulation of ppGpp as well as the preferential inhibition of rRNA synthesis is *relA* independent.

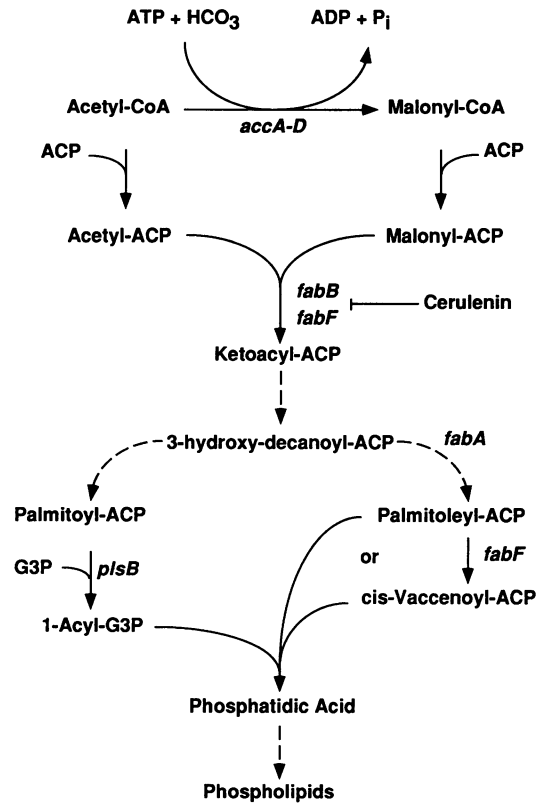

FIG. 1. Synthesis of fatty acids and phospholipids in *E. coli*. *accA–D*, Genes encoding acetyl-CoA carboxylase subunits; *fabB* and *fabF*, genes encoding 3-ketoacyl-[acyl carrier-protein (ACP)] synthases I and II, respectively; *fabA*, gene encoding 3-hydroxydecanoyl-ACP dehydrase; *plsB*, gene encoding *sn*-glycerol-3-phosphate acyltransferase; G3P, glycerol 3-phosphate. Broken arrows indicate multiple steps.

Table 1. Effect of an upward shift in temperature on the relative differential synthesis rate of rRNA in an *accD* ts mutant

Temperature	R_r/R_t rate ratio	
	NO3979 (*accD*$^+$)	NO3978 (*accD*^{ts})
34°C	1.0	0.66 ± 0.03
42°C	1.0	0.34 ± 0.14

Strains were grown at 34°C in AB/glucose medium containing 20 amino acids and thiamine, and the relative differential synthesis rates of rRNA were measured before and 30 min after the temperature shift to 42°C. The values for the mutant are normalized to those for the wild type, and represent averages of three independent experiments.

Effects of Inhibition of Fatty Acid Synthesis by Cerulenin. To explain the above properties of the *accD* mutant, we originally considered two possibilities: first, the acetyl-CoA carboxylase protein might function as a negative regulatory factor of ppGpp synthesis; and second, failure of this enzyme to synthesize fatty acids might indirectly stimulate ppGpp synthesis by an unknown mechanism. To distinguish between these two possibilities, we examined the effect of inhibiting fatty acid synthesis at other, more distal steps in the pathway with respect to ppGpp accumulation and rRNA synthesis inhibition. If inhibiting fatty acid synthesis at one or more distal steps resulted in the same response, then fatty acid starvation rather than inactivation of a regulatory factor would explain the above observations. Cerulenin, a specific inhibitor of fatty acid synthesis (ref. 17; see Fig. 1), was used for this purpose. In *E. coli*, two enzymes, 3-ketoacyl-ACP synthases I and II (encoded by *fabB* and *fabF*, respectively) catalyze the elongation reaction, the former being more sensitive to the inhibitor than the latter. At high concentrations of cerulenin, both enzymes are inhibited, leading to inhibition of both saturated and unsaturated fatty acid synthesis.

Strain NO3980 (*relA*$^+$ *accD*$^+$) was grown in minimal glucose medium at 37°C and treated with cerulenin (100 μg/ml).

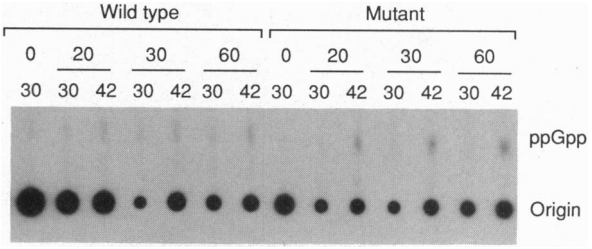

FIG. 2. Increased accumulation of ppGpp in an *accD* ts mutant after the shift up in temperature. Cultures of *accD* ts mutant (NO3978) and its parent (NO3979) were grown at 30°C in AB/glucose medium containing thiamine. At a cell density of 0.15 OD_{600}, part of the cultures was shifted to 42°C (time 0) and the other part was maintained at 30°C (lanes 30 and 42). Samples were taken at the indicated times in minutes (lanes 0, 20, 30, and 60). Equal culture volumes were analyzed for ppGpp by PEI-cellulose TLC, and an autoradiogram is shown. Since the *accD* mutant grew more slowly after the temperature shift, differences between the mutant and the parent in the accumulation of ppGpp per unit cell mass must have been greater than those seen in the autoradiogram. Quantification showed that ppGpp (corrected for cell mass) in the mutant was 4-fold higher than that in the wild type at 42°C.

Accumulation of both stable RNA (Fig. 3*A*) and protein (Fig. 3*B*) was inhibited, the former being more severely affected than the latter. Fig. 3*C* shows that cerulenin treatment caused an increase in the level of ppGpp. This is reminiscent of what is seen during a stringent response caused by amino acid starvation or nutritional deprivation.

In the same experiment, we also measured the cellular concentrations of ATP and ADP (AMP was not detectable) by two-dimensional TLC. A slight decrease (about 25%) in ATP was noted at 15 min after cerulenin treatment, but this difference from the (untreated) control was not seen at 60 min. Calculation of the energy charge {[ATP] + 1/2 [ADP]/([ATP] +[ADP] +[AMP])} also failed to show any significant difference between the control and the cerulenin-treated cultures analyzed at these time points (data not shown; see also lanes 5 and 6 compared with 3 and 4 in Fig. 6 below).

These results show that inhibition of fatty acid synthesis can account for the phenotype observed in the *accD* ts mutant upon a shift up in temperature. In addition, the results appear to exclude the possibility that fatty acid deficiency causes inhibition of stable RNA synthesis and ppGpp accumulation as a result of its (possible) effects on adenylate energy charge.

Inhibition of Unsaturated Fatty Acid Synthesis. Strain CY50 carries a *fabA* ts mutation (ref. 6; see Fig. 1). When temperature is shifted to 42°C, unsaturated fatty acid synthesis stops, while saturated fatty acid synthesis continues in this mutant (6). After the temperature shift, cell mass increases about 4-fold before growth ceases (data not shown). We asked whether the regulatory response—ppGpp accumulation and inhibition of rRNA synthesis—observed with cerulenin requires inhibition of synthesis of both saturated and unsaturated fatty acids or, alternatively, whether deficiency of either one leads to the regulatory response. Both the *fabA*^{ts} and *fabA*$^{+}$ strains were grown at 30°C. After temperature shift to 42°C, the level of ppGpp increased in the *fabA*^{ts} mutant cells but not in the control *fabA*$^{+}$ cells. Addition of an unsaturated fatty acid (palmitoleic acid) to the medium fully prevented this ppGpp accumulation (Fig. 4). Therefore, starvation for unsaturated fatty acids seems to be sufficient to cause ppGpp accumulation.

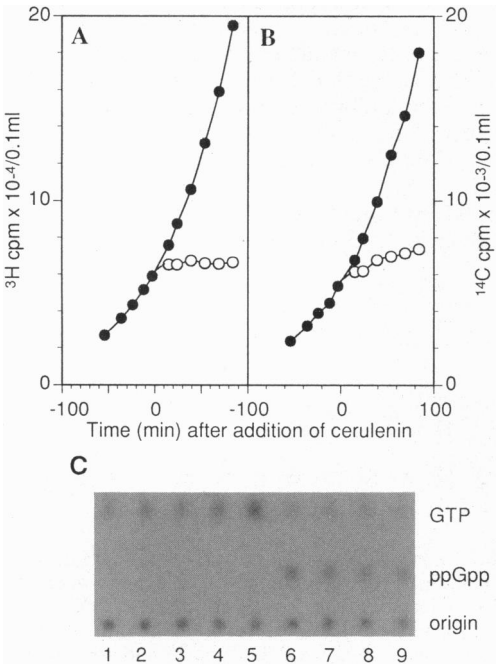

FIG. 3. Effects of cerulenin on the accumulation of RNA (*A*), protein (*B*), and ppGpp (*C*). Strain NO3980 was grown in Mops/glucose medium and treated with cerulenin (100 μg/ml for *A* and *B*, 200 μg/ml for *C*) (time 0). In *C*, an autoradiogram of a PEI-cellulose TLC plate is shown; lanes 6–9 are samples taken at 6, 15, 30, and 60 min after cerulenin addition, respectively, and lanes 2–5 are corresponding samples from the control culture. Lane 1 in *C* is a sample taken 9 min before cerulenin addition.

A different type of experiment was carried out to test whether starvation for saturated fatty acids caused ppGpp accumulation. Cells were treated with cerulenin in the presence of palmitoleic acid, *cis*-vaccenic acid, or both unsaturated fatty acids, thus presumably resulting in a deficiency of only saturated fatty acids. The addition of unsaturated fatty acids only weakly reduced the ppGpp accumulation due to cerulenin (data not shown), suggesting the possibility that starvation for saturated fatty acids can stimulate ppGpp accumulation. However, since we have not succeeded in reversing the cerulenin effect by adding saturated fatty acids (together with unsaturated fatty acids), the role of saturated fatty acids remains to be established by more rigorous experiments.

***spoT* Dependency of ppGpp Synthesis and Stable RNA Repression Caused by Fatty Acid Starvation.** Cashel and his coworkers recently constructed a strain in which both the *relA* and *spoT* genes are deleted and demonstrated the absence of ppGpp in this strain even during carbon starvation (2). We used their system to examine the role of the *spoT* gene in ppGpp accumulation and stable RNA repression caused by fatty acid starvation. Three isogenic strains, *relA*$^{+}$ *spoT*$^{+}$, Δ*relA spoT*$^{+}$, and Δ*relA* Δ*spoT*, were grown in Mops/glucose medium containing 20 amino acids and were treated with cerulenin, after which the ppGpp concentration and differential synthesis rate of rRNA were determined. Cerulenin induced ppGpp accumulation in the two *spoT*$^{+}$ strains but not in the Δ*relA* Δ*spoT* mutant (Fig. 5). Similarly, preferential inhibition of rRNA synthesis was observed only in the wild-type and Δ*relA spoT*$^{+}$ strains and not in the Δ*relA* Δ*spoT* mutant (Table 2). We conclude that fatty acid deficiency caused ppGpp accumulation and preferential inhibition of stable RNA synthesis in *relA*$^{-}$ strains by a *spoT*-dependent mechanism. [It should be noted that fatty acid starvation might also cause ppGpp accumulation via a *relA*-dependent mechanism, perhaps indirectly by somehow causing deficiency of some amino acids. Accumulation of ppGpp induced by cerulenin was greater in the wild-type (MG1655) strain than the Δ*relA spoT*$^{+}$ strain unless all 20 amino acids were supplied in minimal glucose medium. To eliminate compli-

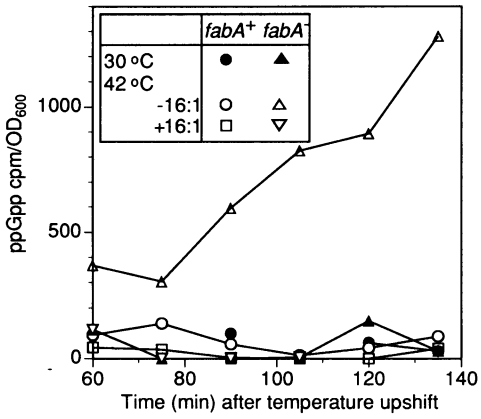

FIG. 4. ppGpp accumulation in a *fabA* ts mutant. Strains KL185 (*fabA*$^{+}$) and CY50 (*fabA*$^{-}$) were grown at 30°C in Mops/glucose medium containing 20 amino acids, uridine, and thiamine, and part of the cultures was subjected to a temperature shift to 42°C with or without addition of 1.8 mM palmitoleic acid (16:1). Symbols are explained in the *Inset*. In other experiments, the accumulation of ppGpp (as well as inhibition of growth) was found to take place slowly in the *fabA*^{ts} mutant after the temperature shift. Therefore, no ppGpp measurements were done before the 60-min time point in the experiment shown.

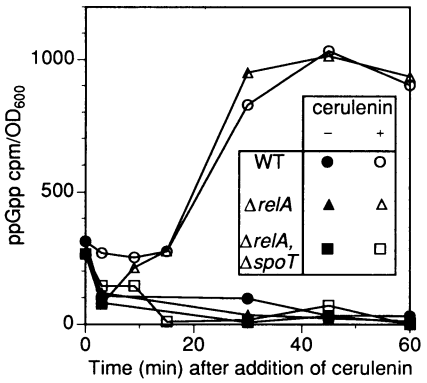

FIG. 5. Cerulenin-stimulated ppGpp accumulation is *spoT* dependent. Strains MG1655 ("WT") and its isogenic derivatives NO3982 (Δ*relA*) and CF1693 (Δ*relA*, Δ*spoT*) were grown in Mops/glucose containing 20 amino acids, and part of the cultures were treated with cerulenin (200 μg/ml). Small amounts of ppGpp calculated to be present in CF1693 are probably due to experimental errors, since visual inspection of the original autoradiogram failed to show any definite ppGpp spot, as reported in ref. 2.

cation due to the *relA*-mediated mechanism, the experiments presented in this paper were carried out either with *relA*$^-$ strains or in the presence of all 20 amino acids except for the experiment shown in Fig. 3.]

Effects of Inhibition of Phospholipid Synthesis. Since fatty acids are the precursors of phospholipids, ppGpp accumulation and inhibition of rRNA synthesis caused by fatty acid starvation might be a result of inhibition of phospholipid synthesis. To examine this possibility, we measured ppGpp levels after direct inhibition of phospholipid synthesis. We used strain NO3983, carrying a mutation in *plsB*, which causes a decrease in the affinity of *sn*-glycerol-3-phosphate acyltransferase (see Fig. 1) for glycerol 3-phosphate (7). As a consequence, it depends on exogenous glycerol to maintain sufficiently high intracellular pools of glycerol 3-phosphate for adequate phospholipid synthesis. When deprived of glycerol 3-phosphate (by filtration and resuspension in glycerol-free medium), phospholipid accumulation immediately stops, and its synthesis rate is also inhibited by at least 90% (7). However, fatty acid synthesis continues for some time (16).

Fig. 6 shows the effect of glycerol removal on growth, phospholipid synthesis, and ppGpp accumulation in the *plsB* mutant, NO3983. While cell mass increased by about 25%

Table 2. Effects of cerulenin on relative [^{3}H]uridine incorporation into total RNA and relative differential rRNA synthesis rates in *relA* and *spoT* deletion mutants

Strains	Cerulenin treatment	Relative [^{3}H]Urd incorp.	Relative R_r/R_t
MG1655	−	1.00	1.00
	+	0.14	0.60
Δ*relA251*	−	1.00	1.00
	+	0.29	0.61
Δ*relA251* Δ*spoT207*	−	1.00	1.00
	+	0.88	1.16

Three isogenic strains, MG1655 (*relA*$^+$, *spoT*$^+$), NO3980 (Δ*relA*) and CF1693 (Δ*relA* Δ*spoT*), were grown in Mops/glucose medium containing 20 amino acids and treated with cerulenin (75 μg/ml). Cells were pulse-labeled with [^{3}H]uridine at 48 min after cerulenin addition. The amounts of [^{3}H]uridine incorporated (incorp.) into total RNA per unit amount of cell mass were determined and then normalized to the values obtained for the controls without cerulenin ("incorporation"). Similarly, the normalized values are shown for the relative differential rRNA synthesis rate (R_r/R_t).

(Fig. 6*A*), phospholipid accumulation ceased immediately (Fig. 6*B*). The rate of phospholipid synthesis as measured by [^{32}P]phosphate pulse-labeling was reduced to 33% at 9 min and further reduced to 15% at 52 min after glycerol removal (Table 3). However, glycerol removal did not cause any increase in the level of ppGpp over the basal level (i.e., of cells growing in the presence of glycerol) for at least 1 hr after glycerol removal (Fig. 6*C*, lanes 7–10 compared with lanes 1–4). To confirm that such phospholipid-starved mutant cells can still accumulate ppGpp when appropriately stimulated, we also inhibited fatty acid synthesis 30 min after glycerol removal by addition of cerulenin. Such treatment increased the level of ppGpp (Fig. 6*C*, lanes 11 and 12), indicating that phospholipid starvation *per se* does not interfere with *spoT*-mediated ppGpp accumulation.

In the above experiments with the *plsB* mutant NO3983, cells growing in the presence of glycerol were also treated with cerulenin. As expected, cerulenin treatment caused accumulation of ppGpp (Fig. 6*C*, lanes 5 and 6), and the rate of phospholipid synthesis was reduced to 12% of the control, comparable to the value (15%) obtained after glycerol removal, a treatment that failed to stimulate accumulation of ppGpp (Table 3). These results show that the cause of ppGpp accumulation (and inhibition of stable RNA synthesis) is deficiency in fatty acid synthesis rather than deficiency in

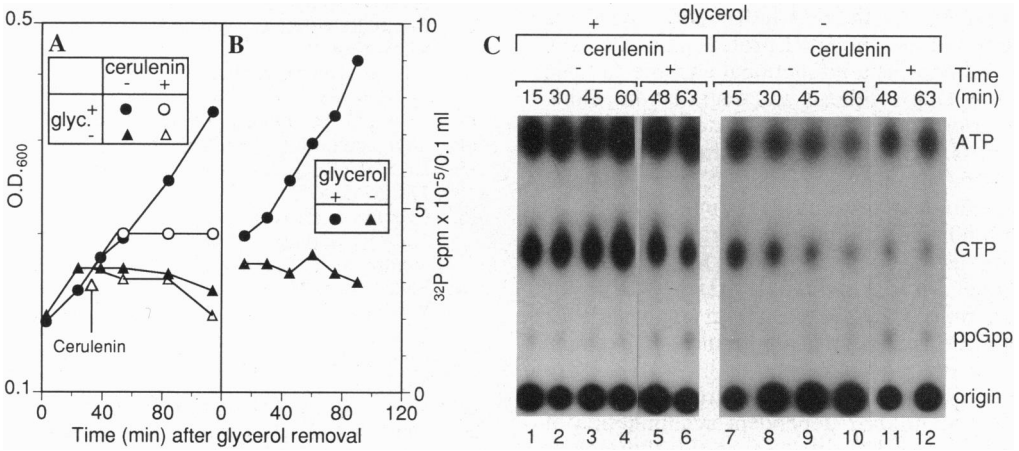

FIG. 6. Effects of glycerol starvation of a *plsB* strain on growth (*A*), phospholipid accumulation (*B*), and ppGpp accumulation (*C*). Strain NO3983 was grown in Mops/glucose medium containing 20 amino acids, glycerol, and [^{32}P]orthophosphate. At time 0, glycerol was removed by filtration, and cells were resuspended in the same medium including [^{32}P]orthophosphate but with (+) or without (−) glycerol. Cerulenin (200 μg/ml) was added to part of the cultures 33 min after glycerol removal, and samples were taken at various times as indicated. *Insets* in *A* and *B* explain symbols. An autoradiogram of a PEI-cellulose TLC plate is shown in *C*.

Table 3. Effects of glycerol starvation of a *plsB* strain on the rate of phospholipid synthesis

Glycerol present (+) or time after removal in min	Cerulenin treatment	Phospholipid synthesis rate	
		cpm × 10^{-3}/min per cells at OD_{600}	Relative to control
+	−	104	1.0
9	−	34	0.33
+	−	155	1.0
52	−	23	0.15
+	+	19	0.12
45	+	6.0	0.04

Strain NO3983 was grown as in Fig. 6 but in the absence of [^{32}P]phosphate; glycerol starvation and cerulenin treatment were also carried out in the same way. At the times indicated, samples were taken and phospholipid synthesis rates were determined. For cerulenin-treated (200 μg/ml, added at 30 min) samples taken at 45 min, the control sample (with glycerol, without cerulenin) at 52 min was used for normalization.

phospholipid synthesis. Using a strain carrying the same *plsB* mutation, Bell previously reported that inhibition of phospholipid synthesis by glycerol deprivation did not lead to simultaneous inhibition of stable RNA synthesis (7), consistent with the conclusion obtained in the present study.

DISCUSSION

We have found that a deficiency in fatty acid supply in *E. coli* leads to accumulation of ppGpp and preferential inhibition of stable RNA synthesis and that these responses to fatty acid deficiency are not due to deficient phospholipid synthesis or insufficient energy supply and are, at least in part, *spoT* dependent. Although growth-rate-dependent regulation of stable RNA synthesis can take place in the absence of ppGpp (18), extensive examples of an inverse correlation between the levels of ppGpp and synthesis rates of stable RNA exist (see ref. 1), as is also the case under conditions of fatty acid starvation described in this paper. Therefore, we restrict our discussion to the accumulation of ppGpp induced by fatty acid starvation and assume that the inhibition of stable RNA synthesis is a result of ppGpp accumulation.

The mechanism of ppGpp synthesis stimulated by fatty acid starvation is unknown. The *spoT* gene was originally identified as the gene encoding an enzyme that degrades ppGpp, ppGpp 3′-pyrophosphohydrolase (reviewed in ref. 1). However, from the analysis of Δ*relA* Δ*spoT* double-deletion mutants, Cashel and his coworkers (2) showed that the *spoT* gene is essential for the *relA*-independent synthesis of ppGpp and suggested that the SpoT protein participates in ppGpp synthesis, perhaps as a bifunctional enzyme capable of catalyzing both ppGpp synthesis and degradation. Thus, one possible scenario to explain the *spoT*-dependent accumulation of ppGpp is that fatty acids (or their derivatives, such as fatty acyl-CoA or fatty acyl-ACP; cf. ref. 19) inhibit the postulated *spoT* function responsible for ppGpp synthesis perhaps by either binding directly to SpoT protein or binding to another positive factor required for the SpoT-catalyzed reaction. Alternatively, it is also possible that fatty acids (or their derivatives) stimulate the SpoT function responsible for ppGpp degradation. Further studies are required to examine the validity of these and other possibilities.

It is well known that carbon (energy source) deprivation leads to *relA*-independent *spoT*-dependent accumulation of ppGpp (2). It is not known how cells sense the shortage of carbon (and energy) source and stimulate ppGpp accumulation. Accumulation of ppGpp during fatty acid starvation takes place in the presence of excess carbon (and energy) source and without a significant decrease in ATP or energy charge, as described in *Results*. Yet, both ppGpp accumulation induced by carbon starvation and that induced by fatty acid starvation are *spoT* dependent. One can consider two possibilities. First, the two phenomena, ppGpp synthesis by carbon starvation and that by fatty acid starvation, may use independent signal transduction mechanisms, although both utilize SpoT protein. Alternatively, carbon starvation might lead to fatty acid starvation, which would be a more direct cause of ppGpp production. According to the second possibility, fatty acid synthesis plays an important function as an indicator of carbon (and energy) sufficiency. In this connection it is interesting to note that certain fatty acids inhibit sporulation of *Bacillus subtilis* (20) and that this might be related to the recent observation of fatty acid inhibition of the KinA protein kinase, which is involved in the initiation of sporulation in *B. subtilis* (21). For this example, as well as for the regulatory phenomenon in *E. coli* described in this paper, depletion of fatty acids correlates with a regulatory response to nutrient deprivation. Thus, the possible (negative) role of fatty acids (or their derivatives) in ppGpp production stimulated by carbon starvation deserves serious future study.

We thank Loan Vu and David Hirota for their technical help in the early stages of this work, Robert Bell and Michael Cashel for mutant strains, and Charles O. Rock and Michael Cashel for critical reading of the manuscript. This work was supported by Public Health Service Grant R37GM35949 and National Science Foundation Grant DMB8904131. M.S. was supported by a fellowship from the University of California, Irvine, Medical Scientist Program.

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