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Inorganic polyphosphate and the induction of *rpoS* expression

TOSHIKAZU SHIBA*†, KAORI TSUTSUMI*, HIROYUKI YANO‡§, YOSHIHARU IHARA*, ATSUSHI KAMEDA*, KAN TANAKA¶, HIDEO TAKAHASHI¶, MASANOBU MUNEKATA*, NARAYANA N. RAO‖, AND ARTHUR KORNBERG‖

*Division of Molecular Chemistry, Graduate School of Engineering, and ‡Department of Chemistry II, Faculty of Science, Hokkaido University, Sapporo 060, Japan; ¶Institute of Molecular and Cellular Biosciences, University of Tokyo, Bunkyo-ku, Tokyo 113, Japan; and ‖Department of Biochemistry, Stanford University School of Medicine, Stanford, CA 94305-5307

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ABSTRACT **Inorganic polyphosphate [poly(P)] levels in *Escherichia coli* were reduced to barely detectable concentrations by expression of the plasmid-borne gene for a potent yeast exopolyphosphatase [poly(P)ase]. As a consequence, resistance to H_2O_2 was greatly diminished, particularly in *katG* (catalase HPI) mutants, implying a major role for the other catalase, the stationary-phase KatE (HPII), which is *rpoS* dependent. Resistance was restored to wild-type levels by complementation with plasmids expressing *ppk*, the gene for PPK [the polyphosphate kinase that generates poly(P)]. Induction of expression of both *katE* and *rpoS* (the stationary-phase σ factor) was prevented in cells in which the poly(P)ase was overproduced. Inasmuch as this inhibition by poly(P)ase did not affect the levels of the stringent-response guanosine nucleotides (pppGpp and ppGpp) and in view of the capacity of additional *rpoS* expression to suppress the poly(P)ase inhibition of *katE* expression, a role is proposed for poly(P) in inducing the expression of *rpoS*.**

Inorganic polyphosphate [poly(P)] is a polymer of tens or hundreds of orthophosphate (P_i) residues linked by high-energy phosphoanhydride bonds (1). Poly(P) accumulates in massive amounts in many bacteria and fungi and in smaller amounts in every microbe, plant, and animal examined (1–3). Potential functions of poly(P) include the following: (*i*) a substitute for ATP for sugar and adenylate kinases (4–6), (*ii*) a phosphate reservoir (1), (*iii*) a chelator for divalent cations (7, 8), (*iv*) a buffer for alkaline stress (9), (*v*) a component in competence for DNA entry and transformation (10, 11), and (*vi*) a factor in regulatory responses to stresses and nutritional deficiencies (12).

An *Escherichia coli* mutant (*ppk*$^-$) that lacks polyphosphate kinase (PPK), the enzyme that makes poly(P), is deficient in functions expressed in the stationary phase and fails to survive (13). The mutant shows a lack of resistance to an oxidant (H_2O_2), to heat, and to an osmotic challenge (12). The heat sensitivity of the *ppk* mutant is suppressed by extra copies of *rpoS*, the gene encoding the stationary-phase-specific RNA polymerase σ factor (12). Expression of *rpoS* is a central element in a regulatory network that governs the expression of many stationary-phase-induced and osmotically regulated genes, including *katE* (14–16). These lines of evidence indicate that poly(P) relates to *rpoS* in its transcription or translation or in stabilization of the RpoS (σ^{38}) protein, inasmuch as the cellular content of σ^{38} is regulated at both the transcriptional and post-transcriptional levels (16).

To elucidate the functions of poly(P) *in vivo*, we have examined how poly(P) might be involved in H_2O_2 resistance by removing poly(P) with a plasmid-borne exopolyphosphatase [poly(P)ase, PPX1] (17). By this approach to achieve a poly(P)-less state, the emergence of resistant variants (12) in the *ppk* mutant can be avoided as well as the supplying of poly(P) by an alternative pathway (11). We report here that the high sensitivity to H_2O_2 was dependent mainly on the *katE* gene product, HPII catalase (18), and that poly(P) is necessary for induction of the transcription not only of *katE* but also of *rpoS*.

MATERIALS AND METHODS

Bacterial Strains and Plasmids. The *E. coli* strains used are as follows: CSH7 (*lacY, rpsL, thi-1*) (18); UM178 (as CSH7 but *katE1, his, lac*$^+$) (18); UM196 (as UM178 but *katG17*::Tn*10*) (18); NY001 (P1 transduction of *katG17*::Tn*10* from UM196 to CSH7); KT1008 (F$^-$, Δ(*arg–lac*)*U169, araD139, rpsL150, ptsF25, flbB5301, rbsR, deoC1*) (19); KT1008EL (as KT1008 but λRS45: *katE–lacZ*) (19); KT1008SL (as KT1008 but λRS45: *rpoS–lacZ*); JM101 {Δ(*lac–proAB*), *supE, thi-1*/F′ [*traD36, proAB*$^+$, *lacI*^{q}, *lacZ*ΔMI5]}; CA10 (as JM101 but *ppk, ppx*) (12).

λ lysogens carrying *katE–lacZ* (KT1008EL) were constructed as described (19), and *rpoS–lacZ* (KT1008SL) was constructed as follows. A DNA fragment corresponding to the 1.4-kb *Cla*I–*Dra*I fragment of the *rpoS* promoter region was inserted into the operon fusion plasmid pRS551 (20), and the *lacZ* fusion construct was subsequently cloned in λRS45 (21). XL1-Blue (Stratagene) was the host strain for plasmid preparations.

The plasmids used are listed in Table 1. Plasmid pTrcPPX1 contains the entire coding region of the yeast *PPX1* gene (1,484-bp *Bam*Hl fragment) and overproduces histidine-tagged PPX1 under control of the *trc* promoter (17); pTrcHisB (Invitrogen) is the original expression vector of pTrcPPX1; pLGPPX1 and pSUPPX1 were constructed by inserting a 3.0-kb *Sph*I–*Bgl*II fragment that contains the *lacI*^{q} gene, *trc* promoter, and *PPX1* gene of pTrcPPX1 into *Sph*I–*Bam*HI double-digested pLG339 (22) and pSU2719 (23), respectively; pLGHisB and pSUHisB are control vectors of pLGPPX1 and pSUPPX1, respectively, that lack the *Bam*HI fragment containing the *PPX1* gene but have the *lacI*^{q} gene and *trc* promoter; pLGPPX1 and pLGHisB are low-copy-number plasmids that are derivatives of pSC105; pSUPPX1 and pSUHisB are medium-copy-number plasmids that are derivatives of pACYC184; pBC29 is a derivative of pUC18 that has the whole region of the *ppk* gene (24); pBS-rpoS, which carries the entire coding region of the *rpoS* gene under the *lac* promoter in pBluescript II SK (+) (Stratagene), was provided by K. Makino (Osaka University, Japan).

Abbreviations: poly(P), inorganic polyphosphate; poly(P)ase, exopolyphosphatase; PPK, poly(P) kinase; HP, hydroperoxidase (catalase); ppGpp, guanosine 5′-diphosphate 3′-diphosphate; pppGpp, guanosine 5′-triphosphate 3′-diphosphate; (p)ppGpp, ppGpp and pppGpp.

†To whom reprint requests should be addressed. e-mail: shiba@moby.hokudai.ac.jp.
§Present address: Laboratory of Molecular Biology, Institute for Chemical Research, Kyoto University, Gokashou, Uji, Kyoto 611, Japan.

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Table 1. Plasmids

Plasmid	Characteristics	Source or ref.
pTrcHisB	Expression vector of His-tagged protein with *lacI*^{q} gene and *trc* promoter; Ap resistance	Invitrogen
PTrcPPX1	pTrHisB derivative carrying *PPX*1 gene; Ap resistance	17
pLGHisB	Expression vector of His-tagged protein with *lacI*^{q} gene and *trc* promoter derived from pSC105; Km resistance	This study
pLGPPX1	pLGHisB derivative carrying *PPX*1 gene; Km resistance	This study
pSUHisB	Expression vector of His-tagged protein with *lacI*^{q} gene and *trc* promoter derived from pACYC184; Cm resistance	This study
pSUPPX1	pSUHisB derivative carrying *PPX*1 gene; Cm resistance	This study
pMM*kat*F3	pBT153 (a pBR322 derivative) with *rpoS* gene; Ap resistance	36
pBC29	pUC18 derivative carrying *E. coli ppk* gene; Ap resistance	24
pBS-rpoS	pBluescript II SK(+) derivative carrying *rpoS* gene; Ap resistance	K. Makino

Ap, ampicillin; Km, kanamycin; Cm, chloramphenicol.

Reagents. Restriction and DNA modification enzymes were obtained from Takara Shuzo (Kyoto) or New England Biolabs. [^{32}P]Orthophosphate ([^{32}P]P_i) was supplied by Amersham. Polyethyleneimine-cellulose thin-layer chromatography (PEI-TLC) plastic plates were from Merck. Anti-RpoS antibody was prepared as described (15). Purified histidine-tagged PPX1 enzyme used in poly(P) estimation was prepared as described (17).

Measurement of H_2O_2 Sensitivity. To cells grown overnight in Luria–Bertani (LB) medium, isopropyl β-D-thiogalactopyranoside (IPTG) was added to a final concentration of 1 mM with a further incubation for 1 hr. The stationary-phase cells were washed and resuspended in 150 mM NaCl to an OD_{600} of 5.0. H_2O_2 was added to a final concentration of 42 mM. At the times indicated, samples were diluted immediately in 150 mM NaCl and spread on LB plates to determine viable cell numbers.

Starvation Conditions for β-Galactosidase Assay and Western Blotting. Exponentially growing cells ($OD_{600} \approx 0.5$) in LB, collected and washed with M9 minimal medium (25) without NH_4Cl and supplemented with glucose (16 mM), 0.2% Casamino acids, and 1 mg/ml thiamin, were concentrated to 1/10 the original culture volume in M9 medium lacking NH_4Cl and starved for several hours at 37°C with shaking. At the times indicated, samples were collected for β-galactosidase assay and Western blotting.

Estimation of Cellular Poly(P). Exponentially growing KT1008 cells harboring the plasmid containing *PPX1* (pTrcPPX1) or the control vector (pTrcHisB) were labeled with [^{32}P]P_i for at least 3 hr in LB until growth reached $OD_{600} \approx 0.5$. The P_i concentration in LB, determined by the method of Chen *et al.* (26), is near 2.4 mM. Cells were washed once, concentrated to 1/10 the original culture volume, and starved in MOPS medium (27) containing glucose (16 mM), K_2HPO_4 (2.4 mM), and [^{32}P]P_i at the same specific activity as that of LB. From cells collected by centrifugation at the times indicated, poly(P) was extracted and estimated by the DEAE filter method (N.N.R., S. Liu, and A.K., unpublished results).

Visualization of Catalases (Hydroperoxidases HPI and HPII) on Acrylamide Gels. Typical HPI and HPII bands were visualized by electrophoresis of whole-cell extracts on an 8% polyacrylamide gel and then stained with a 50:50 solution of 2% $K_3Fe(CN)_6$ and 2% $FeCl_3$. Bovine serum catalase (Sigma) was used as a standard.

Other Procedures. The β-galactosidase assay was performed as described (28). Western blotting and all DNA manipulations

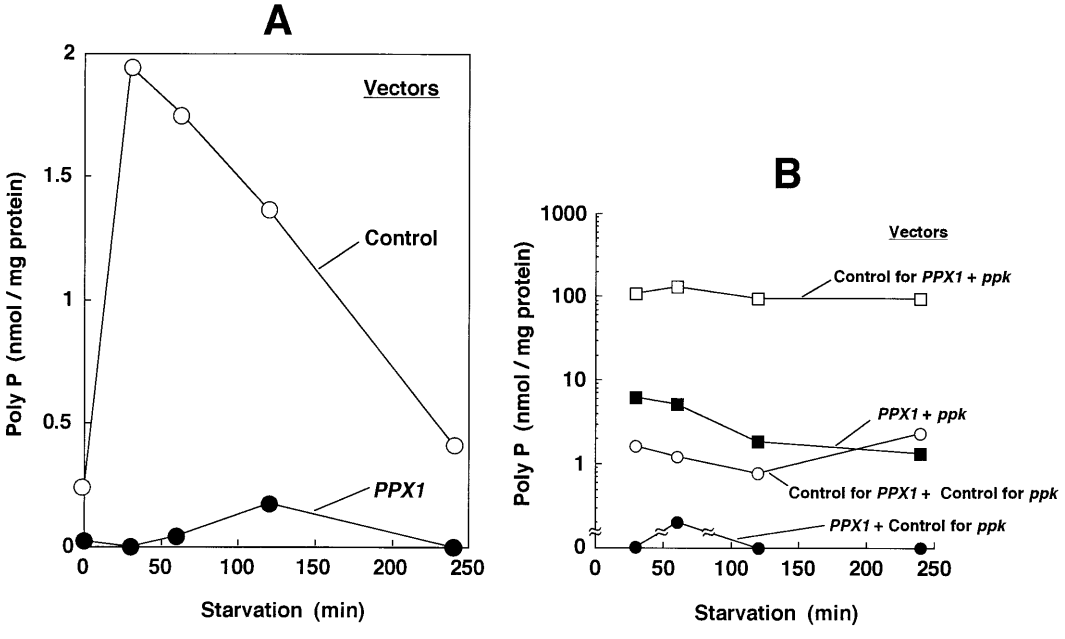

FIG. 1. (*A*) Starvation-induced poly(P) accumulation. KT1008 cells, harboring either the plasmid with the *PPX1* gene (pTrcPPX1) (see text) or the control vector (pTrcHisB) were starved in nitrogen-free MOPS minimal medium (27). (*B*) Poly(P) levels reduced by poly(P)ase overproduction are restored by extra copies of the *ppk* gene. KT1008 cells harbored pairs of plasmids as follows: *PPX1* gene (pLGPPX1) and *ppk* gene (pBC29) (■); control vector for *PPX1* (pLGHisB) and control vector for *ppk* (pUC18) (○); and control vector for *PPX1* (pLGHisB) and *ppk* gene (pBC29) (□). Poly(P) values for cells harboring both the *PPX1* gene (pLG PPX1) and the control vector for *ppk* (pUC18) (●) were below detectable levels (0.1 nmol/mg of protein).

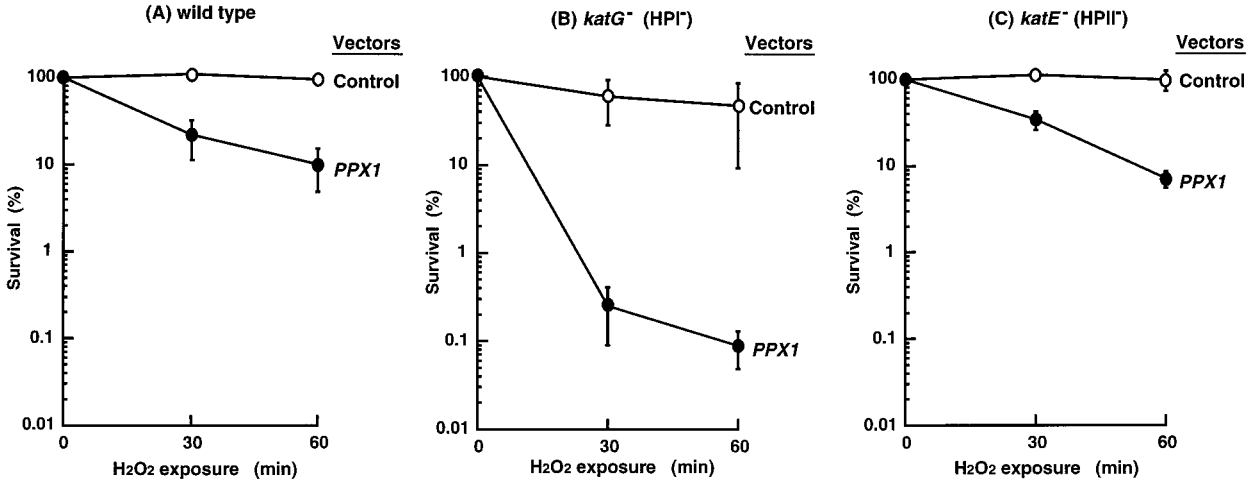

FIG. 2. Effects of poly(P)ase overproduction on H_2O_2 sensitivity in catalase-deficient strains. The wild-type strain (CSH7) in *A*, or the *katG* mutant (NY001) in *B*, or the *katE* mutant (UM178) in *C*, harbored either the plasmid with the *PPX1* gene (pTrcPPX1) (●) or the control vector (pTrcHisB) (○). Cells were exposed to 42 mM H_2O_2 at 25°C; viable cell numbers were determined by plating onto LB agar.

were done by the methods described by Sambrook *et al.* (25). Cellular (p)ppGpp was estimated as described by Manoil and Kaiser (29). Concentrations of poly(P) are given in terms of phosphate residues.

RESULTS

Overproduction of Polyphosphatase Decreases Poly(P) to Barely Detectable Levels, and the Reduced Poly(P) Levels Can Be Restored by the *ppk* Gene. Reduction of poly(P) levels was achieved with a plasmid (pTrcPPX1) that overproduces the yeast exopoly(P)ase (PPX1). After the cells (KT1008) were transformed by either pTrcPPX1 or the corresponding vector plasmid lacking *PPX1* (pTrcHisB), they were cultured to mid-logarithmic phase (OD_{600} = 0.4) and starved for 4 hr as described in *Materials and Methods*. Poly(P) accumulated within the first 30 min of starvation of vector-only-bearing cells and then decreased gradually (Fig. 1*A*). In cells transformed with plasmids containing *PPX1*, poly(P) accumulation was below detectable levels (0.1 nmol/mg of protein).

The reduced poly(P) levels due to poly(P)ase overproduction could be overcome by complementation of the strain with extra copies of the *ppk* gene (Fig. 1*B*). The concentrations of poly(P) were brought down below the detectable limit (0.1 nmol/mg of protein) in a strain that carried a plasmid with *PPX1* and a control vector for *ppk*. When the *ppk* control vector was replaced by the same vector bearing the *ppk* gene in the poly(P)ase-overproducing strain, the levels of poly(P) rose above 1 nmol/mg of protein and fluctuated between 1 and 8 nmol/mg of protein (Fig. 1*B*). A strain that contained control vectors for both *ppk* and *PPX1* accumulated about 1–2 nmol/mg of protein of poly(P), a value similar to the one observed with the control strain in Fig. 1*A*. A strain containing multiple copies of *ppk* and the control vector for *PPX1* accumulated a very high, but constant, amount (about 100 nmol/mg of protein) of poly(P), as expected (Fig. 1*B*).

Table 2. Resistance to H_2O_2 restored by extra copies of the *ppk* gene

Vectors*	Genotype	Viability,† %
Control for *PPX1* + control for *ppk*	Wild type	100
Control for *PPX1* + *ppk*	ppk^{+++}	31
PPX1 + control for *ppk*	$PPX1^{+++}$	0.5
PPX1 + *ppk*	$PPX1^{+++}$ + ppk^{+++}	29

*The plasmids were as follows: control for *PPX1* = pLGHisB, control for *ppk* = pUC18, ppk^{+++} = pBC29, and $PPX1^{+++}$ = pLGPPX1.
†Stationary-phase cells were exposed to 42 mM H_2O_2 for 20 min. Viability with no H_2O_2 exposure is set at 100%.

Poly(P)ase Overproduction Results in Sensitivity to H_2O_2. Just as the *ppk* mutant with low poly(P) levels showed sensitivity to H_2O_2 (12, 13), so does the cell in which poly(P)ase is overproduced by *PPX1* contained in a plasmid (Fig. 2). The 10-fold increase in H_2O_2 sensitivity (Fig. 2*A*) became 1000-fold in a *katG* mutant (lacking the HPI catalase). Both the *katE* mutant (lacking HPII, the stationary-phase-induced catalase) and the wild type show the same levels of sensitivity (Fig. 2 *A* and *C*). Thus, the catalase most dependent on poly(P) in stationary-phase cells is HPII, the only catalase present in *katG* mutant cells (Fig. 2*B*). At the same time, the sharply decreased resistance to H_2O_2 of these cells when deprived of poly(P) is evidence for the contribution by HPII to this resistance.

Although the basal expression of *katG* also depends on the RpoS level in stationary phase (30), the *katG* expression dependent on the OxyR activator may be sufficient to remove

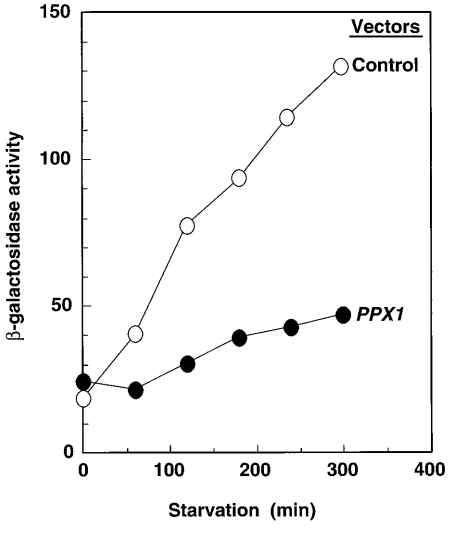

FIG. 3. Expression of the *katE–lacZ* operon fusion monitored by β-galactosidase activity. KT1008EL cells harboring either plasmids with the *PPX1* gene (pTrcPPX1) or the control vector (pTrcHisB) were starved in a M9 minimal medium (25). β-Galactosidase activity was measured in Miller units (28).

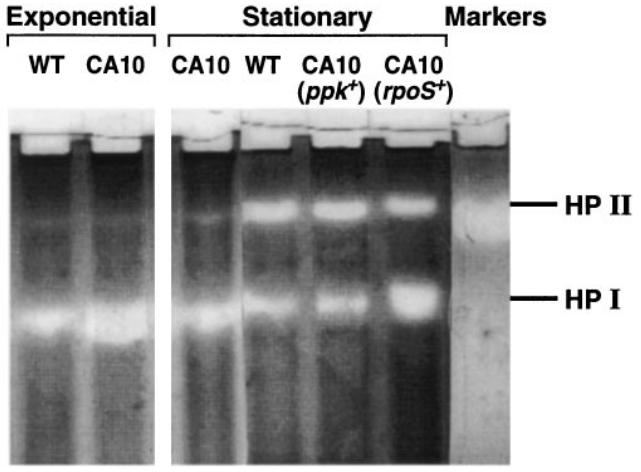

FIG. 4. Visualization of catalase activities (HPI and HPII). Electrophoresis of extracts of wild-type (WT) and CA10 (*ppk*$^-$) cells grown to exponential or stationary phase was performed on an 8.5% acrylamide nondenaturing gel; extracts of stationary-phase CA10 cells complemented with plasmids bearing *ppk* (pBC29) or *rpoS* (pM-M*kat*F3) were also tested.

H_2O_2, thus accounting for the greater H_2O_2 sensitivity of the *katG* mutant at low poly(P) levels (Fig. 2).

Furthermore, resistance to H_2O_2 was also restored in poly(P)ase-overproducing cells to almost the same level as in transformants that harbored both *PPX1*- and *ppk*-bearing plasmids (Table 2). Thus, the heightened sensitivity to H_2O_2 caused by poly(P)ase overproduction can be ascribed to a decreased level of poly(P).

Transcription of *katE* Is Not Induced in Cells Lacking Poly(P). The level of *katE* expression as monitored by β-galactosidase activity with a *katE–lacZ* transcriptional fusion was induced by starvation and reached a maximum value after 5 hr (Fig. 3). However, no significant induction of expression was observed when poly(P)ase was overproduced. Thus, the high resistance to H_2O_2 (Fig. 2, Table 2) is likely due to the dependence of *katE* gene expression on adequate levels of poly(P).

Production of HPII Depends on Poly(P) and *rpoS*. Direct assays of the levels of HPII, the *katE* gene product, demonstrate this catalase to be a stationary-phase-induced enzyme, the induction of which is not observed in *ppk* mutant cells (Fig. 4). This failure can be overcome by supplying the *ppk* gene or, remarkably, by extra copies of *rpoS*.

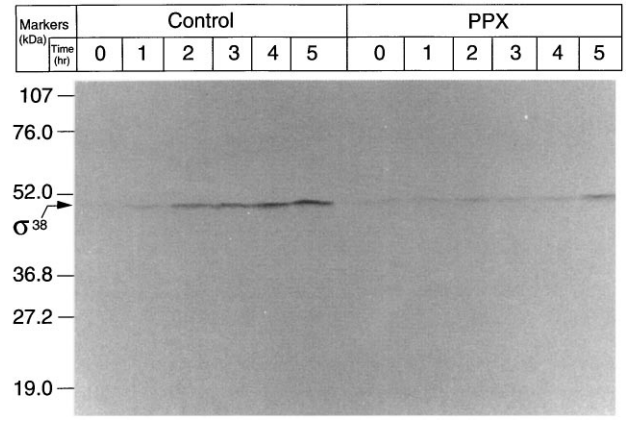

FIG. 5. Levels of RpoS (σ^{38}) in KT1008EL cells harboring plasmids with either the *PPX1* gene or the control vector during starvation. The plasmids used were pTrcPPX1 or pTrcHisB. Proteins were subjected to Western blotting with anti-σ^{38} antiserum and visualized with an alkaline phosphatase-conjugated second antibody.

Cellular Content of RpoS Fails to Increase in Cells Lacking Poly(P). Because *katE* expression is dependent on RpoS, the cellular content of this σ factor (σ^{38}) in a cell overproducing poly(P)ase was determined. As estimated by Western blotting (Fig. 5), the σ^{38} level increased with starvation time in control cells but no significant increase was observed in cells that overproduced poly(P)ase, even after 4 hr of starvation. Because the apparent molecular mass of σ^{38} in this gel is higher than the reported value, we used extracts of both an *rpoS* knockout mutant and an RpoS overproducer as controls, and confirmed that the product is really σ^{38}. Once again, these results suggest that the high sensitivity to H_2O_2 is caused by lack of *rpoS* expression.

Transcription of the *rpoS* Gene in Cells Lacking Poly(P). In a λ lysogen of *E. coli* carrying the *rpoS–lacZ* transcriptional fusion gene, *rpoS* transcription was monitored by β-galactosidase activity (Fig. 6). Cells bearing the poly(P)ase gene or the control vector were grown to mid-logarithmic phase and then starved in a minimal medium. In the vector-only transformants, *rpoS* expression increased with starvation time, reaching a maximum level after 4 hr (Fig. 6*A*) (31). On the other hand, *rpoS* expression was induced only 2-fold after 5 hr of starvation in the transformants lacking poly(P) (Fig. 6*A*).

Expression of *rpoS* was also monitored by β-galactosidase activity in cultures grown in LB (Fig. 6*B*). A vector-only control showed induction of *rpoS* during entry into stationary phase,

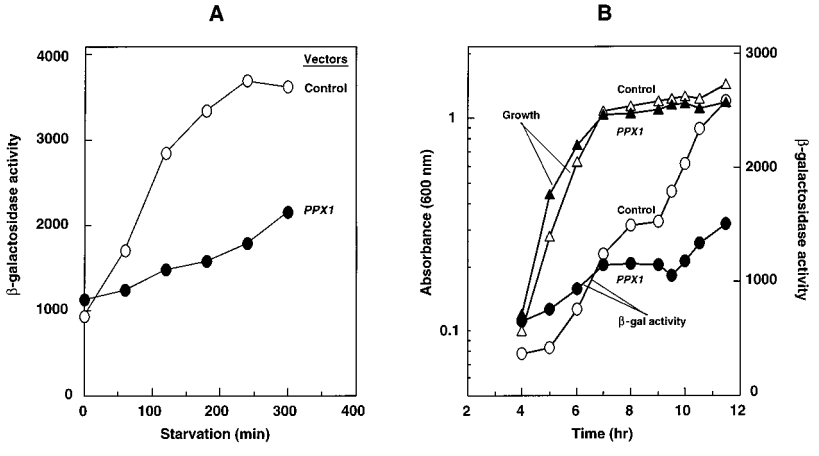

FIG. 6. Expression by the *rpoS–lacZ* operon fusion monitored by β-galactosidase activity (Miller units). KT1008SL cells harbored either plasmids with the *PPX1* gene (pTrcPPX1) or the control vector (pTrcHisB). (*A*) Induction of *rpoS* transcription was monitored after starvation in an M9 minimal medium (25). (*B*) Expression of *rpoS-lacZ* fusion was tested during growth in LB.

with expression increasing nearly 5-fold after 12 hr. In the poly(P)ase-overproducing transformants, the increase in expression was only 2-fold. Inasmuch as growth rates of control and *PPX1* transformants were very much the same, implying no gross differences in metabolism, these results suggest that stationary-phase induction of *rpoS* expression is modulated by poly(P) levels.

Poly(P)ase Overproduction Does Not Affect Accumulation of (p)ppGpp. To see whether the overproduction of poly(P)ase affected the levels of pppGpp and ppGpp, the positive signals for accumulation of σ^{38} (32, 33), the levels of (p)ppGpp were monitored after starvation. Both ppGpp and pppGpp accumulated after 30 min of starvation, and they remained at the same level for 2 hr in the presence of the poly(P)ase overproducer compared with cells that harbored the control vector for *PPX1* (data not shown).

DISCUSSION

Mutants that fail to express *ppk*, the gene that encodes the poly(P) kinase (PPK) responsible for the synthesis of poly(P) in *E. coli*, fail to develop the resistance to heat, oxidants, and osmotic stresses characteristic of stationary-phase cells and lose their viability within a few days (12). A relationship of poly(P) level to the expression of *rpoS*, the σ^{38} factor that controls the induction of some 50 genes in the stationary phase, was observed in the capacity of extra copies of *rpoS* to overcome the deficiencies in the *ppk* mutant (12). However, detailed genetic and physiologic studies of the *ppk* mutant and the regulatory role of poly(P) have been hampered by the genetic instability of the mutant and residual levels of poly(P) presumably synthesized by another route.

To circumvent these difficulties, another approach to deplete the cell of poly(P) was taken in the present studies. Poly(P) levels in cells transformed with a high-copy-number plasmid bearing the gene for the potent yeast exopolyphosphatase [poly(P)ase, PPX1], are reduced to virtually undetectable levels, thus enabling an evaluation of the influence of various genetic backgrounds, transcription efficiencies, and physiologic consequences. The large accumulations of poly(P) that result from overproduction of PPK by a multicopy plasmid bearing *ppk* are markedly reduced by overexpression of poly(P)ase (PPX1) (Fig. 1*B*); plasmid vectors that lacked the *ppk* and *PPX1* genes served as controls.

Sensitivity to H_2O_2 evinced by transformants that overproduce poly(P)ase can be attributed to depressed levels of *katE*, the stationary-phase catalase (HPII) (Fig. 2). The dependence of *katE* on poly(P), as measured by peroxide sensitivity, was 100-fold greater than that of the exponential-phase catalase (HPI), product of the *katG* gene. The relative contributions of these catalases to resistance to peroxide is dependent on cell density, among other factors, and is thus difficult to assess. Under our conditions, the susceptibilities of the *katE* and *katG* mutants to peroxide were no greater than that of the wild type (Fig. 2).

The dependence on poly(P) of the expression of *rpoS* was observed in four ways: (*i*) β-galactosidase activity produced from an *rpoS–lacZ* operon fusion was sharply reduced in cells with overproduced PPX1 (Fig. 6); (*ii*) a similar result was obtained with an *rpoS*-activated gene, as in the *katE–lacZ* operon fusion (Fig. 3); (*iii*) HPII, the catalase product of *katE* expression, was absent from a *ppk* mutant, a defect that could be complemented with either *ppk* or *rpoS* (Fig. 4); and (*iv*) the cellular content of RpoS (σ^{38}) failed to increase upon starvation in cells lacking poly(P) (Fig. 5).

That the low level of induction of *katE* transcription in a poly(P)ase overproducer is due to a decrease in the induction of *rpoS* expression was also demonstrated by the introduction of extra copies of *rpoS* gene on a multicopy plasmid (pBS-rpoS) into a poly(P)ase overproducer. *katE* was induced as fully when cells harbored plasmids for overproduction of both poly(P)ase and *rpoS* as with cells that harbored a plasmid for *rpoS* and a control vector (data not shown). However, no induction was observed in the strain bearing the poly(P)ase gene and a control vector. These results are consistent with observations (12) that the introduction of the *rpoS* gene in a multicopy plasmid into a *ppk* mutant increased its resistance to heat to the wild-type level.

The principal signals for stress responses in *E. coli* are the guanosine tetra- and pentaphosphates [(p)ppGpp], which accumulate in response to deficiencies in phosphate, nitrogen, amino acids, and other nutrients, as well as to undefined factors that operate in starvation and initiation of the stationary phase (32, 33). In the present study, we observed no effect on the levels of (p)ppGpp upon the virtually complete removal of poly(P). Instead, it has been demonstrated that pppGpp, in particular, profoundly inhibits the *E. coli* poly(P)ase, but not the PPK, thus leading to 100- to 1,000-fold accumulations of poly(P) (34). It has also been shown that (p)ppGpp is essential for the induction of *rpoS* expression (32).

On the basis of available evidence, it is clear that the generation of (p)ppGpp is often the primary response to stresses and nutritional deficiencies; accumulations of poly(P) and *rpoS* follow (32). Furthermore, poly(P) appears to figure in the expression of *rpoS* with the resultant activation of the many stationary-phase genes under *rpoS* control. What remains to be determined are the molecular mechanisms by which poly(P) exercises its regulatory role over the induction, maintenance, and actions of *rpoS*. To date, no activating role for poly(P) with reconstituted RNA polymerase systems has been observed.

Although poly(P) complexes of stationary-phase RNA polymerase have been reported, the nature of the holoenzyme, the specificity of gene activation, and details of the reactants are all unclear (35). *E. coli* RNA polymerase holoenzymes reconstituted from the core polymerase and a σ factor (e.g., RpoD, RpoS) showed no specificity in activating a stationary-phase gene (e.g., *katE*) (H. Wurst and A.K., unpublished results). In fact, the RpoS holoenzyme was uniquely and profoundly inhibited by substoichiometric amounts of a long-chain poly(P) (about 700 residues). Many reasons can be imagined for failures under *in vitro* conditions, among them, the involvement of other factors (e.g., cAMP), DNA topology, and especially the lack of a putative poly(P)-binding protein, which could have been stripped from the core polymerase early in the course of its isolation from the crude cell extracts.

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