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Effects of σ^S and the Transcriptional Activator AppY on Induction of the *Escherichia coli hya* and *cbdAB-appA* Operons in Response to Carbon and Phosphate Starvation

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The transcriptional regulation of two energy metabolism operons, *hya* and *cbdAB-appA*, has been investigated during carbon and phosphate starvation. The *hya* operon encodes hydrogenase 1, and the *cbdAB-appA* operon encodes cytochrome *bd*-II oxidase and acid phosphatase, pH 2.5. Both operons are targets for the transcriptional activator AppY. In exponential growth, expression of the *hya* and *cbd* operons was reduced in an *rpoS* mutant lacking the RNA polymerase σ^S factor, and the induction of the two operons by entry into stationary phase in rich medium was strongly dependent on σ^S. Both operons were induced by carbon starvation, but only induction of the *hya* operon was dependent on σ^S, whereas that of the *cbd* promoter was dependent on AppY. The *appY* gene also showed σ^S-dependent induction by carbon starvation. The *cbd* and *hya* operons were also found to exhibit a σ^S-dependent transient twofold induction by osmotic upshift. Like the *cbd* operon, the *hya* operon was highly induced by phosphate starvation. For both operons the induction was strongly dependent on AppY. The induction ratio of the two operons was the same in *rpoS*$^+$ and *rpoS* mutant strains, indicating that the phosphate starvation-induced increase in σ^S concentration is not involved in the phosphate regulation of these operons.

Escherichia coli is a facultative anaerobe and is able to obtain energy through both fermentation and respiration. *E. coli* synthesizes two terminal oxidases under aerobic growth conditions: cytochrome *o* oxidase and cytochrome *d* oxidase. Both oxidases catalyze the oxidation of ubiquinol-8 and the reduction of oxygen to H_2O as the terminal step in the aerobic respiration chain (for a review, see reference 28). The *cyo* operon, encoding cytochrome *o* oxidase, is maximally expressed during aerobic conditions, whereas the *cyd* operon, encoding cytochrome *d* oxidase, is induced under semianaerobic conditions. Both operons are repressed during anaerobiosis (33). A putative third cytochrome oxidase of *E. coli* has been identified. It is encoded by the two genes located upstream of *appA* (encoding acid phosphatase, pH 2.5). The deduced gene products show strong sequence homology to cytochrome *d* oxidase (11). Recently, it was shown that these genes encode a cytochrome *bd* type oxidase (30), and it was suggested that they should be named *cbdAB* instead of the preliminary gene designation *appCB* by Dassa et al. (11) or *cyxAB* by Atlung and Brøndsted (2). The *cbd* operon is transcribed from promoter p_{cbd} located immediately upstream of *cbdA* (11). The expression of the operon is induced by anaerobiosis, by phosphate starvation, and upon entry into stationary phase (2, 10). The anaerobic induction is dependent on two transcriptional activators (2, 8): (i) the ArcA response regulator (18), which is activated by the ArcB sensor in response to reduced respiration (17, 19), and (ii) the AppY protein, which is a member of the AraC family of transcriptional activators (3, 12). Growth phase induction is also strongly dependent on these two activators (2, 8), and induction by phosphate starvation is severely reduced in an *appY* mutant (2).

appY was originally identified as a gene which when present on a multicopy plasmid strongly stimulated expression of the *appA* gene (3). Expression of the *appY* gene is itself induced by anaerobiosis, entry into stationary phase, and phosphate starvation (8), indicating that the increased AppY concentration under these conditions contributes to the induction of the *cbd* promoter.

The only other operon that has been identified as a target for the AppY activator is the *hya* operon, which is located immediately upstream of the *cbdAB-appA* operon (2). The *hya* operon encodes hydrogenase 1 (23), which can catalyze the uptake and oxidation of H_2 to produce protons and electrons. Like the *cbd* operon, the anaerobic induction of the *hya* operon is dependent on the two activators ArcA and AppY (7).

Transcription from the *cbd* promoter has been shown to be reduced in an *rpoS* (*appR190*) mutant in stationary phase in rich medium and under anaerobic conditions (11), suggesting that this promoter belongs to the class of promoters which can be transcribed by both σ^{70}- and σ^S-containing RNA polymerase (27, 31, 32). Also, the stationary-phase induction of *appY* expression is reduced in an *rpoS* mutant (8). The amounts of σ^S present in *E. coli* is strongly influenced by environmental factors. It increases upon entry into stationary phase in rich medium (22) or starvation for carbon (13, 22) or nitrogen and phosphate (13), and it is also strongly increased by osmotic upshift (25).

Here we show that transcription of the *hya* operon is induced in stationary phase in rich medium by carbon and phosphate starvation and is reduced in an *rpoS359* mutant. We have compared the expression of the *hya* and *cbd* operons in *rpoS*$^+$ and *rpoS359* strains under the different conditions known to affect σ^S levels. To elucidate the contribution of AppY, we determined the effects on *appY* expression in parallel and in-

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TABLE 1. *E. coli* K-12 strains

Strain	Genotype[a]	Reference or construction
MC4100	*araD139* Δ(*argF-lac*)*U169 deoC1 flb5301 relA1 rpsL150 ptsF25 rbsR*	Silhavy et al. (29)
RH90	*araD139* Δ(*argF-lac*)*U169 deoC1 flb5301 relA1 rpsL150 ptsF25 rbsR rpoS359*::Tn*10*	Lange and Hengge-Aronis (21)
HO440	*metB purE zbb-2419*::Tn*10 supF relA spoT rpsL*	Hove-Jensen (16)
TC3594	*thi-1 leu-6 lacY1 lacI-ZΔ*(*Mlu*) *supE44 tonA21 rpsL rfbD1* Δ(*appY-ent*)	Atlung and Brøndsted (2)
TC3981	*thi-1 leu-6 lacY1 lacI-ZΔ*(*Mlu*) *supE44 tonA21 rpsL rfbD1 attB*::p_{cbd}′*-lacZ*	Atlung and Brøndsted (2)
TC3983	*thi-1 leu-6 lacY1 lacI-ZΔ*(*Mlu*) *supE44 tonA21 rpsL rfbD1 attB*::p_{appY}′*-lacZ*	Brøndsted and Atlung (8)
TC3985	*thi-1 leu-6 lacY1 lacI-ZΔ*(*Mlu*) *supE44 tonA21 rpsL rfbD1 attB*::p_{hya}′*-lacZ*	Brøndsted and Atlung (7)
LB56	*purE zbb-2419*::Tn*10*[b]	HO440(P1) × MC4100[c]
LB57	Δ(*appY-ent*)[b]	TC3594(P1) × LB56[d]
LB58	Δ(*appY-ent*) *rpoS359*::Tn*10*[b]	RH90(P1) × LB57[e]
LB130	*attB*::p_{cbd}′*-lacZ*[b]	Brøndsted and Atlung (8)
LB131	*attB*::p_{appY}′*-lacZ*[b]	Brøndsted and Atlung (8)
LB132	*attB*::p_{hya}′*-lacZ*[b]	Brøndsted and Atlung (7)
LB133	*attB*::p_{cbd}′*-lacZ rpoS359*::Tn*10*[b]	TC3981(P1) × RH90[f]
LB134	*attB*::p_{appY}′*-lacZ rpoS359*::Tn*10*[b]	Brøndsted and Atlung (8)
LB135	*attB*::p_{hya}′*-lacZ rpoS359*::Tn*10*[b]	TC3985(P1) × RH90[f]
TC4280	*attB*::p_{cbd}′*-lacZ* Δ(*appY-ent*)[b]	TC3981(P1) × LB57[f]
TC4281	*attB*::p_{hya}′*-lacZ* Δ(*appY-ent*)[b]	TC3985(P1) × LB57[f]
TC4282	*attB*::p_{cbd}′*-lacZ* Δ(*appY-ent*) *rpoS359*::Tn*10*[b]	TC3981(P1) × LB58[f]
TC4283	*attB*::p_{hya}′*-lacZ* Δ(*appY-ent*) *rpoS359*::Tn*10*[b]	TC3985(P1) × LB58[f]

[a] Genetic symbols are according to the method of Bachmann (5).
[b] Genotype otherwise like that of MC4100.
[c] The *purE* mutation was introduced by selecting for tetracycline resistance and testing for a requirement of adenine for growth.
[d] The Δ(*appY-ent*) mutation was introduced by selecting for PurE$^+$ and assaying for low levels of acid phosphatase (pH 2.5) in LB medium overnight cultures as described elsewhere (3).
[e] The *rpoS359*::Tn*10* mutation was introduced by selecting for tetracycline resistance and testing for low levels of catalase activity (21).
[f] The *lacZ* fusion was introduced by selecting for the 100% linked kanamycin resistance gene.

cluded *appY* and *appY rpoS* double mutants in the studies of the *hya* and *cbd* operons.

MATERIALS AND METHODS

Bacterial strains, growth media, and enzyme assays. The strains used for physiological experiments in this study are all derivatives of strain MC4100 (29) and are listed together with the other strains in Table 1. For growth phase experiments cultures were grown in Luria-Bertani (LB) medium (24) with vigorous shaking. For carbon starvation experiments we used AB minimal medium (9) supplemented with 1 μg of thiamine per ml and 0.1% glucose. Phosphate starvation experiments were carried out in morpholinepropanesulfonic acid (MOPS) minimal medium (26) containing 0.132 mM K_2PO_4 supplemented with 1 μg of thiamine per ml and 0.1% glucose. Antibiotics were added at 50 μg/ml (kanamycin) and 20 μg/ml (tetracycline). Cell density was monitored, and β-galactosidase activity (Miller units) was determined as described previously (7). For the phosphate starvation experiments the values given in Table 4 were determined from a differential plot of β-galactosidase activity versus cell density (optical density at 450 nm) to circumvent the problems of inactivation of β-galactosidase due to acidification late in starvation (see Fig. 3). All the experiments were done at least twice. The data presented are from one experiment in which all the cultures were grown at the same time and in the same batch of medium. The results from duplicate experiments were within 30% of the values shown.

RESULTS

Effect of an *rpoS* mutation on stationary-phase induction of *hya* operon expression. In an initial experiment we wanted to examine if expression of the *hya* operon is induced in stationary phase, like that of the *cbd* operon and the *appY* gene, and to test the possible effect of σ^S on *hya* operon expression. To compare the responses of the three promoters, isogenic *rpoS*$^+$ and *rpoS359* derivatives carrying the single-copy p_{cbd}′*-lacZ*, p_{hya}′*-lacZ*, and p_{appY}′*-lacZ* operon fusions were grown in LB medium, and the specific β-galactosidase activity was determined in the exponential growth phase and 3 h after entry into stationary phase (Table 2).

The p_{cbd}′*-lacZ* fusion was very weakly expressed in exponential phase and was induced approximately 20-fold in stationary phase. The *rpoS* mutation had no effect on expression in exponential growth, but stationary-phase expression was reduced 10-fold, in agreement with the previous data from primer extension experiments (11). The p_{hya}′*-lacZ* fusion was also strongly induced in stationary phase (30-fold). The inactivation of *rpoS* had a moderate effect on *hya* expression in exponential phase (4-fold reduction) and a more pronounced effect (20-fold) in stationary phase. Both fusions still showed a weak stationary-phase induction in the absence of σ^S. This might be due to the increase, albeit very moderate, in *appY* expression in stationary phase (Table 2).

These experiments indicate that the *hya* operon, like the *cbdAB* operon, is stationary phase induced and belongs to the RpoS-dependent genes. The stationary-phase induction may be due primarily to the increased concentration of σ^S in cells entering stationary phase.

Induction of *cbd*, *hya*, and *appY* expression by carbon starvation. The concentration of σ^S increases upon carbon starvation (13, 22), and many genes identified as carbon starvation inducible are σ^S dependent (21, 34). We therefore investigated the carbon starvation inducibility of the three *lacZ* fusions (Fig. 1). In the wild-type strain, the p_{cbd}′*-lacZ* fusion was weakly induced and the p_{appY}′*-lacZ* fusion was moderately induced

TABLE 2. Effects of *rpoS359* mutation on stationary-phase induction[a]

Fusion[b]	Sp act of β-galactosidase (U $ml^{-1} \times A_{450}^{-1}$)			
	Exponential phase		Stationary phase	
	rpoS$^+$	*rpoS359*	*rpoS*$^+$	*rpoS359*
p_{cbd}′*-lacZ*	0.04	0.04	0.7	0.07
p_{hya}′*-lacZ*	0.23	0.06	6.0	0.3
p_{appY}′*-lacZ*	2.1	2.2	7.0	4.4

[a] Cells were grown exponentially at 37°C in LB medium, and β-galactosidase activity was determined as described in Materials and Methods.
[b] See Table 1 for complete genotypes.

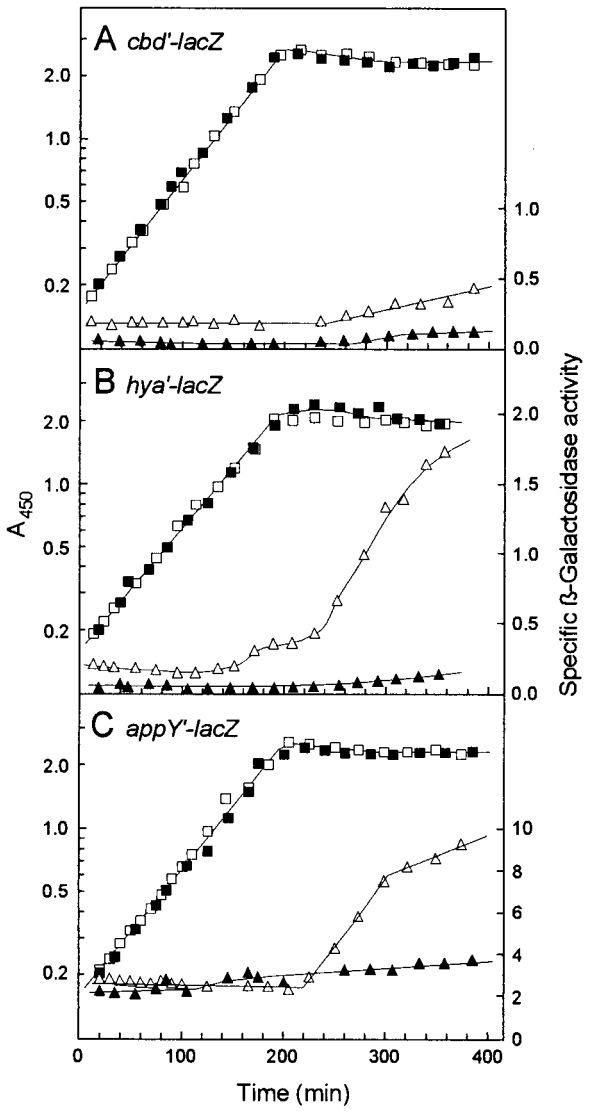

FIG. 1. Carbon starvation induction of *cbd*, *hya*, and *appY* expression in wild-type and *rpoS* mutant strains. The strains were grown at 37°C with good aeration (120 rpm) in AB minimal medium supplemented with 0.1% glucose. Growth of the cultures was monitored spectrophotometrically (squares), and specific β-galactosidase activity (triangles) was determined as described in Materials and Methods. (A) Open symbols, strain LB130 (p_{cbd}'-*lacZ rpoS*$^+$); closed symbols, LB133 (p_{cbd}'-*lacZ rpoS359*). (B) Open symbols, strain LB132 (p_{hya}'-*lacZ rpoS*$^+$); closed symbols, LB135 (p_{hya}'-*lacZ rpoS359*). (C) Open symbols, strain LB131 (p_{appY}'-*lacZ rpoS*$^+$); closed symbols, LB133 (p_{appY}'-*lacZ rpoS359*).

whereas the p_{hya}'-*lacZ* fusion was strongly induced by glucose starvation (Fig. 1 and Table 3). In the *rpoS* mutant, p_{cbd} expression was reduced both in exponential growth and upon carbon starvation but was induced threefold upon glucose starvation like in the wild type (Fig. 1 and Table 3). In contrast, the starvation induction of *appY* and *hya* expression was reduced significantly in the *rpoS* mutant (Fig. 1 and Table 3).

Since carbon starvation caused induction of the *appY* gene and therefore presumably an increase in AppY protein concentration, we investigated the carbon starvation response of the p_{cbd}'-*lacZ*, and p_{hya}'-*lacZ* fusions in *appY* mutants (but not the p_{appY}'-*lacZ* fusion, since *appY* is not autoregulated [8]). The weak induction of the p_{cbd}'-*lacZ* fusion was dependent on AppY in both the absence and presence of RpoS, whereas

TABLE 3. Effects of *rpoS* and *appY* on induction by carbon starvation[a]

Strain	Relevant genotype			Sp act of β-galactosidase (U ml^{-1} × A_{450}^{-1})[b]		Fold regulation[c]
	Fusion	*rpoS*	*appY*	Exponential phase	Starvation phase	
LB130	p_{cbd}'-*lacZ*	+	+	0.17	0.4	3
LB133		−	+	0.04	0.12	3
TC4280		+	−	0.16	0.20	1
TC4282		−	−	0.06	0.07	1
LB132	p_{hya}'-*lacZ*	+	+	0.13	1.7	13
LB135		−	+	0.04	0.11	3
TC4281		+	−	0.08	1.6	20
TC4283		−	−	0.05	0.11	2
LB131	p_{appY}'-*lacZ*	+	+	2.4	9.5	4
LB134		−	+	2.4	3.8	1.5

[a] Strains carrying the indicated fusions (see Table 1 for full genotypes) were grown exponentially at 37°C in AB minimal medium supplemented with 0.1% glucose.
[b] β-Galactosidase activity was determined as described in Materials and Methods. The exponential-phase value is from a differential plot, and the starvation-phase value was taken at 3 h after exhaustion of glucose.
[c] Specific activity in starvation phase/specific activity in exponential phase.

induction of the p_{hya}'-*lacZ* fusion was completely independent of AppY (Table 3).

Effect of osmotic upshift on expression of the *cbd* and *hya* operons. The expression of many σ^S-controlled genes is stimulated in response to an increase in medium osmolarity (14), and recently it was shown that the concentration of σ^S increases upon an osmotic upshift (25). We found that the steady-state expression of the p_{cbd}'-*lacZ* and p_{hya}'-*lacZ* fusions was virtually unchanged by an increase in medium osmolarity (data not shown). An osmotic upshift has no effect on *appY* expression (4). Analysis of the kinetics after an osmotic upshift, however, revealed that both fusions were transiently induced for approximately one generation and that the induction was RpoS dependent (Fig. 2). The period coincides with that where σ^S synthesis and stability are very high (25) and thus the σ^S concentration is maximal. Some time after the osmotic upshift σ^S synthesis and stability decrease somewhat again (25). This close correlation between *cbd* and *hya* expression and σ^S levels strongly suggests that σ^S is participating directly in transcription from the *cbd* and *hya* promoters.

Role of RpoS in induction of the *cbd* and *hya* operons by phosphate starvation. The *cbd* promoter is strongly induced by starvation for inorganic phosphate, and the induction is reduced in an *appY* mutant (2) (see Table 4). The AppY-dependent stimulation of the *cbd* promoter might be mediated by increased levels of AppY protein, since *appY* expression is also strongly induced by phosphate starvation (8) (see Table 4). Expression of the *hya* operon, like that of *cbd*, is stimulated by an increased AppY concentration (1). It was therefore not surprising that the p_{hya}'-*lacZ* fusion was strongly induced by phosphate starvation (Table 4; Fig. 3). The induction was nearly as strongly dependent on AppY as that of the *cbd* promoter (Table 4).

For both the *cbd* and the *hya* fusions there is, however, still a considerable induction in the *appY* mutant, 15- and 40-fold, respectively. The levels of σ^S have been shown to increase to the same degree upon carbon and phosphate starvation (13). We therefore tested whether RpoS was involved in the phosphate starvation induction of the *cbd* and *hya* operons. Inacti-

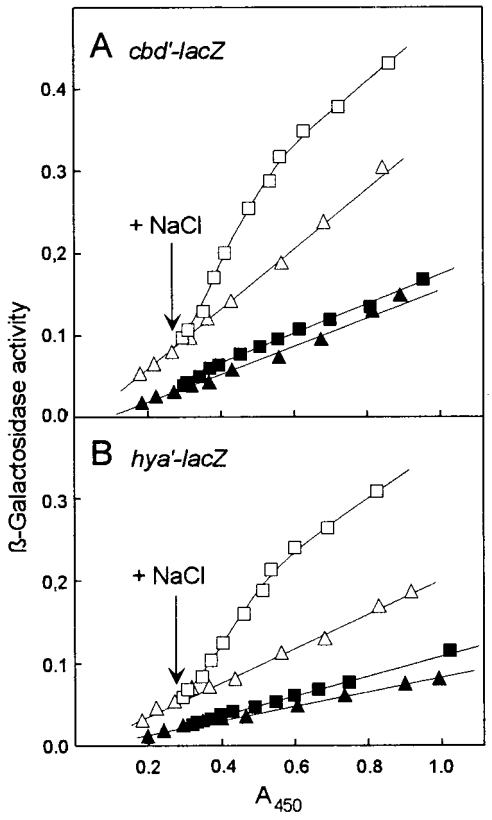

FIG. 2. RpoS-dependent transient induction upon osmotic upshift of *cbd* and *hya* expression. The strains were grown at 37°C with good aeration (120 rpm) in AB minimal medium supplemented with 0.1% glucose. At an A_{450} of approximately 0.25 (arrow), the cultures were divided in two parts, and one part received NaCl to 0.25 M. Triangles, cultures without NaCl; squares, cultures with 0.25 M NaCl. Growth of the cultures was monitored spectrophotometrically, and β-galactosidase activity was determined as described in Materials and Methods. β-Galactosidase activity per milliliter was plotted against A_{450} to emphasize the kinetics of the transient induction. (A) Open symbols, strain LB130 (p_{cbd}'-*lacZ rpoS*$^+$); closed symbols, LB133 (p_{cbd}'-*lacZ rpoS359*). (B) Open symbols, strain LB132 (p_{hya}'-*lacZ rpoS*$^+$); closed symbols, LB135 (p_{hya}'-*lacZ rpoS359*).

vation of *rpoS* in the *appY*$^+$ background decreased the level of β-galactosidase in the starved cultures significantly (Fig. 3). The induction ratios were, however, only slightly affected, since the *rpoS* mutation also reduced expression before starvation (Table 4). In the *appY* mutant background inactivation of *rpoS* had no effect on the prestarvation expression but reduced the induction ratio twofold. The *rpoS* mutation had no effect on induction of the *appY* fusion, eliminating the possibility of an indirect effect through AppY levels.

DISCUSSION

Here we have shown that the expression of both the *cbd* and the *hya* operons is reduced in the absence of σ^S under all growth conditions tested, i.e., during exponential growth, entry into stationary phase in rich medium, and carbon and phosphate starvation and upon osmotic upshift. In contrast, *appY* gene expression was affected only by the absence of σ^S during entry into stationary phase and carbon starvation. The correspondence between the transient (large) increase in σ^S concentration (25) and the transient induction of *hya* and *cbd* during osmotic upshift suggests that σ^S affects expression of these operons directly. The effect of the *rpoS* mutation seen under the other conditions is therefore probably also direct.

Although σ^S always affects the level of expression of *hya* and *cbd*, it is only in some cases that we consider σ^S to have a regulatory role, i.e., the cases where the induction by environmental stimuli is reduced in the *rpoS* mutant. These results have been summarized in Fig. 4. In the wild-type background the increase in σ^S seems to be instrumental in the carbon starvation induction of the *hya* operon and the *appY* gene, and the osmotic induction, albeit small, of both *hya* and *cbd*. The large increase in σ^S upon phosphate starvation (13) did not contribute to the induction of the *hya* or the *cbd* operon in the wild-type strain; only in the *appY* mutant was a small (twofold) effect unmasked. This result indicates that in the absence of AppY protein both promoters might be more efficient with σ^S-containing RNA polymerase than with σ^{70} RNA polymerase. Previously, it has been shown that three of four *rpoS*-dependent carbon starvation-inducible (*csi*) fusions were also induced by phosphate starvation (34), but in these cases the *rpoS* dependence of the P_i starvation induction was not determined. In the only other study of the role of σ^S in phosphate starvation induction, i.e., that of the *cbpA* gene (35), it was found that the induction was dependent on an intact *rpoS* gene. The *cbpA* promoter contains curved DNA and is repressed by H-NS protein (36), whereas the *cbd* and *hya* promoter regions neither exhibit curvature nor bind H-NS (4). In contrast to the *csi* fusions and the *hya* operon, the *cbpA* gene was not induced by carbon starvation (35).

Although *hya* expression and *cbd* expression were very similarly affected by inactivation of *rpoS* and *appY* during phosphate starvation, these two promoter regions responded quite differently to carbon starvation: stimulation of the *hya* transcription was completely dependent on RpoS and independent of AppY, while the opposite was found for the *cbd* promoter (Fig. 4). The AppY-dependent induction of the *hya* and *cbd* operons during anaerobiosis and phosphate starvation seems to be mediated by a combination of increased *appY*

TABLE 4. Effects of *rpoS* and *appY* on induction by phosphate starvation[a]

Strain	Fusion	*rpoS*	*appY*	Sp act of β-galactosidase ($U\ ml^{-1} \times A_{450}^{-1}$)[b]		Fold regulation[c]
				Exponential phase	Phosphate starvation	
LB130	p_{cbd}'-*lacZ*	+	+	0.16	35	220
LB133		−	+	0.11	15	140
TC4280		+	−	0.05	0.8	16
TC4282		−	−	0.05	0.4	8
LB132	p_{hya}'-*lacZ*	+	+	0.14	24	170
LB135		−	+	0.07	13	185
TC4281		+	−	0.06	2.5	40
TC4283		−	−	0.06	1.1	18
LB131	p_{appY}'-*lacZ*	+	+	2.5[d]	54[d]	22
LB134		−	+	2.5[d]	55[d]	22

[a] Strains carrying the indicated fusions (see Table 1 for full genotypes) were grown exponentially at 37°C in MOPS minimal medium supplemented with 0.1% glucose and limiting amounts of phosphate.

[b] The specific β-galactosidase activity was determined from a differential plot of β-galactosidase activity versus the cell density before (exponential phase) and after (P_i starvation) the decrease in growth rate.

[c] Specific activity in starvation phase/specific activity in exponential phase.

[d] Previously published data (8).

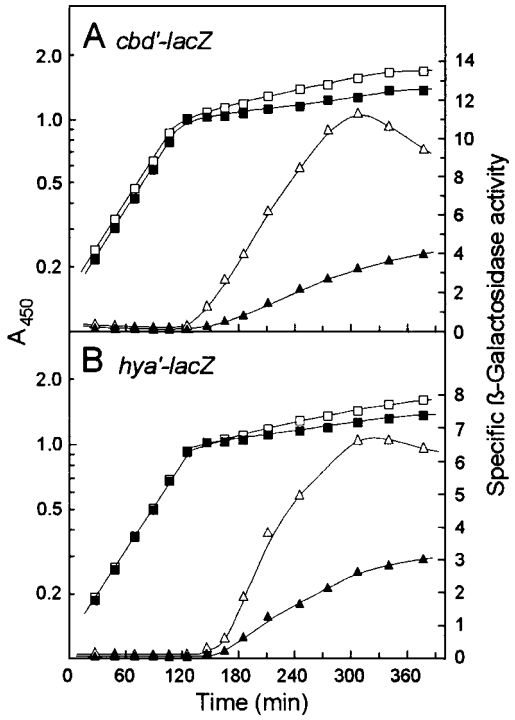

FIG. 3. Induction of *cbd* and *hya* operon expression by phosphate starvation in wild-type and *rpoS* mutant strains grown at 37°C in MOPS minimal medium supplemented with 0.1% glucose and limiting amounts of inorganic phosphate. Growth of the cultures was monitored spectrophotometrically (squares), and β-galactosidase specific activity (triangles) was determined as described in Materials and Methods. (A) Open symbols, strain LB130 (p_{cbd}'-*lacZ rpoS*$^+$); closed symbols, LB133 (p_{cbd}'-*lacZ rpoS359*). (B) Open symbols, strain LB132 (p_{hya}'-*lacZ rpoS*$^+$); closed symbols, LB135 (p_{hya}'-*lacZ rpoS359*).

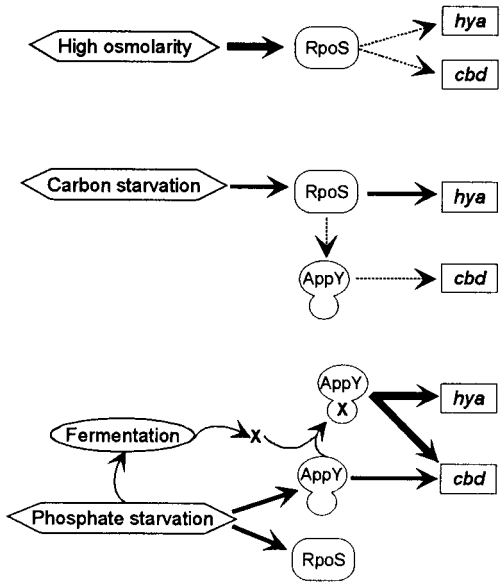

FIG. 4. Comparison of the stimulatory effects of different environmental stimuli on *cbd, hyaI*, and *appY* expression. Fat arrows, strong stimulation of expression (>10-fold); medium arrows, medium stimulatory effects (5- to 10-fold); dotted arrows, weak stimulatory effects (2- to 5-fold); curved arrows, reactions; X, unknown low-molecular-weight effector for AppY. The presumed signal transduction pathway for activation of AppY is included in the phosphate starvation section.

expression and an activating signal for AppY generated under both these conditions (1). The *cbd* promoter seems to be stimulated quite efficiently by increased AppY protein under non-activating conditions (1), while the *hya* promoter is primarily stimulated by activated AppY protein (6). The signal is probably an increased concentration of a metabolite from fermentation (1) and would thus be absent during carbon starvation.

The *hya* promoter responded strongly to the increased σ^S concentration during carbon starvation, whereas the *cbd* promoter seemed completely unresponsive, although the effects of an osmotic upshift were very similar. This difference could be due to the different effects on supercoiling: increased osmolarity leads to increased supercoiling (15), while glucose starvation leads to decreased supercoiling (20). It is possible that initiation of transcription from the *hya* promoter(s) with σ^S RNA polymerase is insensitive to the carbon starvation-induced change in linking number, while that from the *cbd* promoter is inhibited. Alternatively, there might be some difference in posttranslational modification of σ^S under the two conditions which affects the two promoters differently.

The results presented here show that in the wild type approximately two-thirds of the expression of *hya* and *cbd*, both in exponential growth and during phosphate starvation, must arise from initiations with σ^S RNA polymerase. The AppY protein is required for strong stimulation upon phosphate starvation both in the wild type and in the *rpoS* mutant, strongly suggesting that AppY is able to stimulate initiation of transcription with both σ^S- and σ^{70}-containing RNA polymerase.

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REFERENCES

1. **Atlung, T.** 1996. Unpublished results.
2. **Atlung, T., and L. Brøndsted.** 1994. Role of the transcriptional activator AppY in regulation of the *cyx appA* operon of *Escherichia coli* by anaerobiosis, phosphate starvation, and growth phase. J. Bacteriol. **176:**5414–5422.
3. **Atlung, T., A. Nielsen, and F. G. Hansen.** 1989. Isolation, characterization, and nucleotide sequence of *appY*, a regulatory gene for growth-phase-dependent gene expression in *Escherichia coli*. J. Bacteriol. **171:**1683–1691.
4. **Atlung, T., S. Sund, K. Olesen, and L. Brøndsted.** 1996. The histone-like protein H-NS acts as a transcriptional repressor for expression of the anaerobic and growth phase activator AppY of *Escherichia coli*. J. Bacteriol. **178:**3418–3425.
5. **Bachmann, B. J.** 1990. Linkage map of *Escherichia coli* K-12, edition 8. Microbiol. Rev. **54:**130–197.
6. **Brøndsted, L.** 1996. Unpublished results.
7. **Brøndsted, L., and T. Atlung.** 1994. Anaerobic regulation of the hydrogenase 1 (*hya*) operon of *Escherichia coli*. J. Bacteriol. **176:**5423–5428.
8. **Brøndsted, L., and T. Atlung.** 1996. Effect of growth conditions on expression of the acid phosphatase (*cyx-appA*) operon and the *appY* gene, which encodes a transcriptional activator of *Escherichia coli*. J. Bacteriol. **178:**1556–1564.
9. **Clark, D. J., and O. Maaløe.** 1967. DNA replication and the division cycle in *Escherichia coli*. J. Mol. Biol. **23:**99–112.
10. **Dassa, E., M. Cahu, B. Desjoyaux-Cherel, and P. L. Boquet.** 1982. The acid phosphatase with optimum pH of 2.5 of *Escherichia coli*: physiological and biochemical study. J. Biol. Chem. **257:**6669–6676.
11. **Dassa, J., H. Fsihi, C. Marck, M. Dion, M. Kieffer-Bontemps, and P. L. Boquet.** 1991. A new oxygen-regulated operon in *Escherichia coli* comprises the genes for a putative third cytochrome oxidase and for pH 2.5 acid phosphatase (*appA*). Mol. Gen. Genet. **229:**341–352.
12. **Gallegos, M.-T., C. Michán, and J. L. Ramos.** 1993. The XylS/AraC family of regulators. Nucleic Acids Res. **21:**807–810.

13. **Gentry, D. R., V. J. Hernandez, L. H. Nguyen, D. B. Jensen, and M. Cashel.** 1993. Synthesis of the stationary-phase sigma factor σ^S is positively regulated by ppGpp. J. Bacteriol. **175:**7982–7989.
14. **Hengge-Aronis, R., R. Lange, N. Henneberg, and D. Fischer.** 1993. Osmotic regulation of *rpoS*-dependent genes in *Escherichia coli*. J. Bacteriol. **175:**259–265.
15. **Higgins, C. F., C. J. Dorman, D. A. Stirling, L. Waddell, I. R. Booth, G. May, and E. Bremer.** 1988. A physiological role for DNA supercoiling in the osmotic regulation of gene expression in *S. typhimurium* and *E. coli*. Cell **52:**569–584.
16. **Hove-Jensen, B.** 1989. Phosphoribosylpyrophosphate (PRPP)-less mutants of *Escherichia coli*. Mol. Microbiol. **3:**1487–1492.
17. **Iuchi, S., V. Chepuri, H.-A. Fu, R. B. Gennis, and E. C. C. Lin.** 1990. Requirement for terminal cytochromes in generation of the aerobic signal for the *arc* regulatory system in *Escherichia coli*: study utilizing deletions and *lac* fusions of *cyo* and *cyd*. J. Bacteriol. **172:**6020–6025.
18. **Iuchi, S., and E. C. C. Lin.** 1988. *arcA* (*dye*), a global regulatory gene in *Escherichia coli* mediating repression of enzymes in aerobic pathways. Proc. Natl. Acad. Sci. USA **85:**1888–1892.
19. **Iuchi, S., Z. Matsuda, T. Fujiwara, and E. C. C. Lin.** 1990. The *arcB* gene of *Escherichia coli* encodes a sensor regulator protein for anaerobic repression of the *arc* modulon. Mol. Microbiol. **4:**715–727.
20. **Jensen, P. R., L. Loman, B. Petra, C. Vanderweijden, and H. V. Westerhoff.** 1995. Energy buffering of DNA structure fails when *Escherichia coli* runs out of substrate. J. Bacteriol. **177:**3420–3426.
21. **Lange, R., and R. Hengge-Aronis.** 1991. Identification of a central regulator of stationary phase gene expression in *Escherichia coli*. Mol. Microbiol. **5:**49–59.
22. **Lange, R., and R. Hengge-Aronis.** 1994. The cellular concentration of the σ^S subunit of RNA polymerase in *Escherichia coli* is controlled at the levels of transcription, translation, and protein stability. Genes Dev. **8:**1600–1612.
23. **Menon, N. K., J. Robbins, H. D. Peck, Jr., C. Y. Chatelus, E.-S. Choi, and A. E. Przybyla.** 1990. Cloning and sequencing of a putative *Escherichia coli* [NiFe] hydrogenase-1 operon containing six open reading frames. J. Bacteriol. **172:**1969–1977.
24. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
25. **Muffler, A., D. D. Traulsen, R. Lange, and R. Hengge-Aronis.** 1996. Post-transcriptional osmotic regulation of the σ^S subunit of RNA polymerase in *Escherichia coli*. J. Bacteriol. **178:**1607–1613.
26. **Neidhardt, F. C., P. L. Bloch, and D. F. Smith.** 1974. Culture medium for enterobacteria. J. Bacteriol. **119:**736–747.
27. **Nguyen, L. H., D. B. Jensen, N. E. Thompson, D. R. Gentry, and R. R. Burgess.** 1993. In vitro functional characterization of overproduced *Escherichia coli katF*/*rpoS* gene product. Biochemistry **32:**11112–11117.
28. **Poole, R. K., and W. J. Ingledew.** 1987. Pathways of electrons to oxygen, p. 170–200. *In* F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology. American Society for Microbiology, Washington, D.C.
29. **Silhavy, T. J., M. L. Berman, and L. W. Enquist.** 1984. Experiments with gene fusions. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
30. **Sturr, M. G., T. A. Krulwich, and D. B. Hicks.** 1996. Purification of a cytochrome *bd* terminal oxidase encoded by the *Escherichia coli app* locus from a Δ*cyo* Δ*cyd* strain complemented by genes from *Bacillus firmus* OF4. J. Bacteriol. **176:**1742–1749.
31. **Tanaka, K., S. Kusano, N. Fujita, A. Ishihama, and H. Takahashi.** 1995. Promoter determinants for *Escherichia coli* RNA polymerase holoenzyme containing σ^{38} (the *rpoS* gene product). Nucleic Acids Res. **23:**827–834.
32. **Tanaka, K., Y. Takayanagi, N. Fujita, A. Ishihama, and H. Takahashi.** 1993. Heterogeneity of the principal σ factor in *Escherichia coli*: the *rpoS* gene product, σ^{38}, is a second principal σ factor of RNA polymerase in stationary-phase *Escherichia coli*. Proc. Natl. Acad. Sci. USA **90:**3511–3515.
33. **Tseng, C.-P., J. Albrecht, and R. P. Gunsalus.** 1996. Effect of microaerophilic cell growth conditions on expression of the aerobic (*cyoABCDE* and *cydAB*) and anaerobic (*narGHJI*, *frdABCD*, and *dmsABC*) respiratory pathway genes in *Escherichia coli*. J. Bacteriol. **178:**1094–1098.
34. **Weichart, D., R. Lange, N. Henneberg, and R. Hengge-Aronis.** 1993. Identification and characterization of stationary phase-inducible genes in *Escherichia coli*. Mol. Microbiol. **10:**407–420.
35. **Yamashino, T., M. Kakeda, C. Ueguchi, and T. Mizuno.** 1994. An analogue of the DnaJ molecular chaperone whose expression is controlled by σ^S during the stationary phase and phosphate starvation in *Escherichia coli*. Mol. Microbiol. **13:**475–483.
36. **Yamashino, T., C. Ueguchi, and T. Mizuno.** 1995. Quantitative control of the stationary phase-specific sigma factor, σ^S, in *Escherichia coli*: involvement of the nucleoid protein H-NS. EMBO J. **14:**594–602.