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Metabolic Roles of a *Rhodobacter sphaeroides* Member of the σ^{32} Family

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We report the role of a gene (*rpoH*) from the facultative phototroph *Rhodobacter sphaeroides* that encodes a protein (σ^{37}) similar to *Escherichia coli* σ^{32} and other members of the heat shock family of eubacterial sigma factors. *R. sphaeroides* σ^{37} controls genes that function during environmental stress, since an *R. sphaeroides* ΔRpoH mutant is ~30-fold more sensitive to the toxic oxyanion tellurite than wild-type cells. However, the ΔRpoH mutant lacks several phenotypes characteristic of *E. coli* cells lacking σ^{32}. For example, an *R. sphaeroides* ΔRpoH mutant is not generally defective in phage morphogenesis, since it plates the lytic virus RS1, as well as its wild-type parent. In characterizing the response of *R. sphaeroides* to heat, we found that its growth temperature profile is different when cells generate energy by aerobic respiration, anaerobic respiration, or photosynthesis. However, growth of the ΔRpoH mutant is comparable to that of a wild-type strain under each of these conditions. The ΔRpoH mutant mounted a heat shock response when aerobically grown cells were shifted from 30 to 42°C, but it exhibited altered induction kinetics of ~120-, 85-, 75-, and 65-kDa proteins. There was also reduced accumulation of several presumed heat shock transcripts (*rpoD* P_{HS}, *groESL*$_1$, etc.) when aerobically grown ΔRpoH cells were placed at 42°C. Under aerobic conditions, it appears that another sigma factor enables the ΔRpoH mutant to mount a heat shock response, since either RNA polymerase preparations from an ΔRpoH mutant, reconstituted Eσ^{37}, or a holoenzyme containing a 38-kDa protein (σ^{38}) each transcribed *E. coli* Eσ^{32}-dependent promoters. The lower growth temperature profile of photosynthetic cells is correlated with a difference in heat-inducible gene expression, since neither wild-type cells or the ΔRpoH mutant mount a typical heat shock response after such cultures were shifted from 30 to 37°C.

The heat shock response is a universal phenomenon that allows cells to survive a multitude of environmental stresses (28). This response is characterized by a rapid, transient increase in the rate of synthesis of a highly conserved set of polypeptides known collectively as heat shock proteins (HSPs) (14, 28). While increased expression of HSPs is often seen after a stress, most of these gene products are present at significant levels in steady-state cells to promote protein synthesis, folding, and intracellular localization (28). Molecular chaperone function for HSPs has been demonstrated in key cellular processes such as DNA replication, cell division, or maintenance of active protein conformations during conditions that cause cytoplasmic stress (13, 14, 28, 43).

A molecular picture for what triggers the heat shock response is beginning to emerge. In the enteric bacterium *Escherichia coli*, an alternative sigma factor (σ^{32}) that recognizes heat shock gene promoters (15) increases in abundance, stability, and activity under conditions that cause the accumulation of misfolded cytoplasmic proteins (38). Use of alternate sigma factors could be a common way to control the eubacterial heat shock response, since proteins related to *E. coli* σ^{32} have been identified from a diverse group of proteobacteria (4, 24, 27, 32, 41). These other σ^{32} family members are believed to function in much the same manner as *E. coli* σ^{32}, becoming more abundant or active in response to thermal or metabolic stimuli that produce cytoplasmic stress.

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While control of the heat shock response by *E. coli* σ^{32} is considered the eubacterial paradigm, other ways to regulate procaryotic HSP synthesis exist. An additional alternate sigma factor (σ^{E}) recognizes the *E. coli* σ^{32} gene (*rpoH*) and several other heat shock promoters in response to signals that generate periplasmic stress (11). In several other eubacteria, a *cis*-active inverted repeat called CIRCE (for controlling inverted repeat of chaperone expression) negatively regulates heat shock gene expression (1, 19, 46). Indeed, recent experiments suggest that individual *Bradyrhizobium japonicum* heat shock genes are regulated by both members of the σ^{32} family and CIRCE elements (1, 27).

This work sought to identify the function of a member of the σ^{32} family from the facultative phototroph *Rhodobacter sphaeroides*. Previous experiments implicated a 37-kDa *R. sphaeroides* protein (σ^{37}) that reacted with antibody against *E. coli* σ^{32} as a member of the RpoH family (16). The observation that several *E. coli* heat shock promoters were transcribed by *R. sphaeroides* RNA polymerase samples that contained this 37-kDa protein supported the provisional designation of σ^{37} as a member of the σ^{32} family (16). The recent finding that *R. sphaeroides* Eσ^{37} transcribes a promoter (*cycA* P1) for an essential component of the *R. sphaeroides* photosynthetic apparatus like cytochrome c_2 suggested that σ^{37} might have functions outside its commonly accepted role in HSP synthesis (21). Since little is known about the ability of σ^{32} family members to recognize genes other than those which encode HSPs, we were particularly interested in asking if Eσ^{37} or its target genes contribute to expression or assembly of proteins that function in biological energy generation by *R. sphaeroides*.

MATERIALS AND METHODS

Bacterial strains, plasmids, and growth conditions. *E. coli* (Table 1) strains were grown at 37°C in Luria-Bertani medium (33). *R. sphaeroides* strains (Table 1) were routinely grown at 30°C in Sistrom's succinate-based minimal medium A (37). For growth of cells by anaerobic respiration in the dark, dimethyl sulfoxide (DMSO) was used as a terminal electron acceptor in Sistrom's minimal medium containing 20 mM glucose and 0.2% yeast extract (7). Plating efficiency of phage RS1 was determined with aerobically grown cells (6).

Tellurite sensitivity was tested by diluting aerobically grown cells ($\sim 10^9$ cells/ml) sufficiently to produce ~100 colonies per plate (23). To score for growth phenotypes associated with loss of σ^{37}, solid media were used routinely. Where indicated, growth curves with liquid cultures were performed to test phenotypes associated with the Δ*rpoH* allele.

Isolation of *R. sphaeroides rpoH*. An *E. coli* ΔRpoH mutant containing the R40 mutation (CAG12517 [44]) was used to isolate an *R. sphaeroides* gene that recognizes heat shock promoters. While *E. coli* ΔRpoH strains are unable to grow above 20°C, the R40 mutation allows growth of this strain up to 40°C (17). To identify genes that restore phage growth to CAG12517, this tester strain was infected with a lytic *R. sphaeroides* NCIB8253 λgt11 library (40) at a multiplicity of infection of 0.05.

Chromosomal mapping and Southern blot analysis. Restricted *R. sphaeroides* genomic DNA was separated with a CHEF-DR II apparatus (Bio-Rad Laboratories, Richmond, Calif.) (39). For Southern blot analysis (33), restricted *R. sphaeroides* DNA was transferred to membranes, hybridized with nick-translated ^{32}P-labeled *rpoH* probes (Gibco-BRL, Gaithersburg, Md.), washed at moderate stringency (two 5-min washes at room temperature with a solution consisting of 1× SSPE [1× SSPE is 0.18 M NaCl, 10 mM NaH_2PO_4, and 1 mM EDTA {pH 7.7}] and 0.1% sodium dodecyl sulfate [SDS] and two 15-min washes at 45°C with 0.1× SSPE–0.1% SDS), exposed to a PhosphorImaging screen, and visualized with ImageQuant software (Molecular Dynamics, Sunnyvale, Calif.).

DNA sequence analysis. DNA sequencing used either *Taq* DNA polymerase and deazanucleotide triphosphates (Promega, Inc., Madison, Wis.) or automated sequencers at the University of Wisconsin Madison Biotechnology Center. A list of plasmids and primers used to sequence the ~1.5-kb segment of *R. sphaeroides* DNA in pPJR19 is available upon request. DNA sequences containing *rpoH* were analyzed with software from the University of Wisconsin Genetics Computer Group, Madison, Wis. (5).

Construction of a ΔRpoH mutant. To produce an *R. sphaeroides* ΔRpoH mutant, a *Sty*I restriction fragment internal to *rpoH* was replaced with a spectinomycin resistance (Sp^r) gene. Specifically, pPJR19 was digested with *Sty*I, and the ends were made blunt with T4 DNA polymerase and ligated with a 2-kb *Sma*I restriction fragment carrying an omega (Ω) cartridge encoding Sp^r (31). The resultant plasmid (pPJR26) was digested with *Apa*I to produce a restriction fragment containing the interrupted (Δ*rpoH1*::ΩSp^r) gene. After the ends of this restriction fragment were made blunt, it was purified and cloned into pSUP202, a mobilizable suicide vector (36), which had been digested with *Pst*I and *Eco*RI and treated with T4 DNA polymerase. The resulting plasmid (pPJR29) was used to place a Δ*rpoH1*::ΩSp^r allele in *R. sphaeroides* 2.4.1 (7). Screening Sp^r cells (25 μg/ml) for those sensitive to tetracycline (1 μg/ml) identified a strain (RpoH1) where the Δ*rpoH1*::ΩSp^r allele replaced a wild-type gene by homologous recombination (8). One Tc^r strain (RpoH26), in which Δ*rpoH1*::ΩSp^r was integrated into the chromosome along with the suicide plasmid, was used to aid genomic mapping of *rpoH* (see Results).

Rates of protein synthesis. Rates of protein synthesis were determined before and after log-phase aerobically grown *R. sphaeroides* cultures ($\sim 10^9$ cells/ml) were shifted from 30 to 42°C. At indicated times, 1-ml samples were removed, labeled with 50 μCi of [^{35}S]Transmet (Amersham Corp., Arlington Heights, Ill.) for 1 min, and chased with a mixture of 1 M cysteine and 1 M methionine for 2 min. At this time, cold trichloroacetic acid was added to a final concentration of 5%, proteins were harvested by centrifugation (13,000 × *g*, 10 min), the supernatant was aspirated, and the precipitate was washed twice with ice-cold 80% acetone. After evaporation of residual liquid under vacuum, 100 μl of solubilization buffer containing 10% β-mercaptoethanol was added (33). Samples of equal radioactivity were separated by SDS–12.5% polyacrylamide gel electrophoresis prior to analysis and quantitation on a PhosphorImager with ImageQuant software (Molecular Dynamics).

For monitoring the rates of protein synthesis under photosynthetic conditions, cells were grown to mid-exponential phase ($\sim 5 \times 10^8$ CFU/ml) at 30°C in 15-ml screw-capped tubes at a light intensity of 10 W/m^2. Protein synthesis was monitored as described above.

Primer extension assays. RNA from cells grown aerobically (45) was used in primer extension assays (2) with these promoter-specific oligonucleotides: *rpoD* P_{HS}, 5′-CCTCGACCGCCTCCTCGATTTCCT-3′ (3a); *groESL*$_1$, 5′-CACGGTCATGCAGCGTTTG-3′ (19); *rrnB*, 5′-AAGACAAAACAAACCGAGACGCCA-3′ (10). Products were separated on denaturing polyacrylamide gels (2) prior to estimation of their levels on a PhosphorImager with ImageQuant software.

In vitro transcription assays. Conditions for preparation of *R. sphaeroides* RNA polymerase, reconstitution of core enzyme samples with potential sigma factors, and their use for in vitro transcription assays with plasmid templates have been described previously (21).

TABLE 1. Bacterial strains and plasmids

Strain or plasmid	Relevant feature(s)	Source or reference
R. sphaeroides		
2.4.1	Wild type	Laboratory strain
RpoH1	Δ*rpoH1*::ΩSp^r derivative of 2.4.1	This work
RpoH26	Δ*rpoH1*::ΩSp^r derivative of 2.4.1 with pPJR19 integrated	This work
E. coli		
DH5α	Strain used for plasmid maintenance	3
S17-1	Conjugative donor for mating	36
CAG12517	MC4100 (R40) Δ*rpoH30*::*kan*, suppressor tRNA	44
Plasmids		
pGEM-7Zf(+)	Ap^r	Promega
pSUP202	Ap^r Tc^r Cm^r *R. sphaeroides* suicide plasmid	36
pHP45Ω	Ap^r Sp^r source of 2-kb ΩSp^r cartridge	31
pPJR18	4.3-kb *Eco*RI restriction fragment of *rpoH* in pGEM-7Zf(+)	This work
pPRJ19	pPJR18 lacking 3-kb *Sma*I restriction fragment	This work
pPJR26	pPJR19 *Sty*I restriction fragment replaced by ΩSp^r cartridge	This work
pPJR29	*rpoH* region from pPRJ26 in pSUP202	This work

Nucleotide sequence accession number. DNA sequences containing *rpoH* were deposited with accession no. U82397.

RESULTS

Identification of *R. sphaeroides rpoH*. *E. coli* ΔRpoH mutants are unable to support λ growth because they are limited for the HSPs that are required for phage DNA replication (13). To identify a gene product that supports λ infection, an *E. coli* ΔRpoH strain was infected with an *R. sphaeroides* genomic library in the lytic λgt11 vector (40). Phage DNA was purified (33), digested with *Eco*RI to yield a 4.3-kb restriction fragment, and the DNA was cloned into pGEM-7Zf(+) to yield pPJR18. To confirm that heat shock gene expression was dependent on *R. sphaeroides* DNA, this plasmid was shown to support 44°C growth and confer λ sensitivity to an *E. coli* ΔRpoH mutant (data not shown). In addition, a plasmid (pPJR19) containing a smaller, ~1.5-kb, restriction fragment (Table 1) activated an *rpoD* P_{HS}::*lacZ* fusion (data not shown). The following experiments indicate that the responsible *R. sphaeroides* gene (*rpoH*) encodes a sigma factor related to proteins in the eubacterial σ^{32} family.

***R. sphaeroides* RpoH is a member of the σ^{32} family.** The *R. sphaeroides* DNA that activates *E. coli* heat shock gene expression encodes a 298-amino-acid protein (33.7 kDa) whose putative start codon (coordinate 418 in accession number U82397) begins 10 bases downstream of a potential ribosome binding site (AGAGG). The deduced protein has a high degree of amino acid similarity to eubacterial proteins in the σ^{32} family (Fig. 1); it displays 68% identity to *Caulobacter crescentus* σ^{32} (32, 41), 66% identity to *Agrobacterium tumefaciens* σ^{32} (26), 58% identity to *B. japonicum* σ^{32} (27), and 40% identity to *E. coli* σ^{32} (15, 18). The similarity of *R. sphaeroides* RpoH to σ^{32} family members extends throughout the protein, but the considerable conservation in regions 4.2 (−35 recognition) and

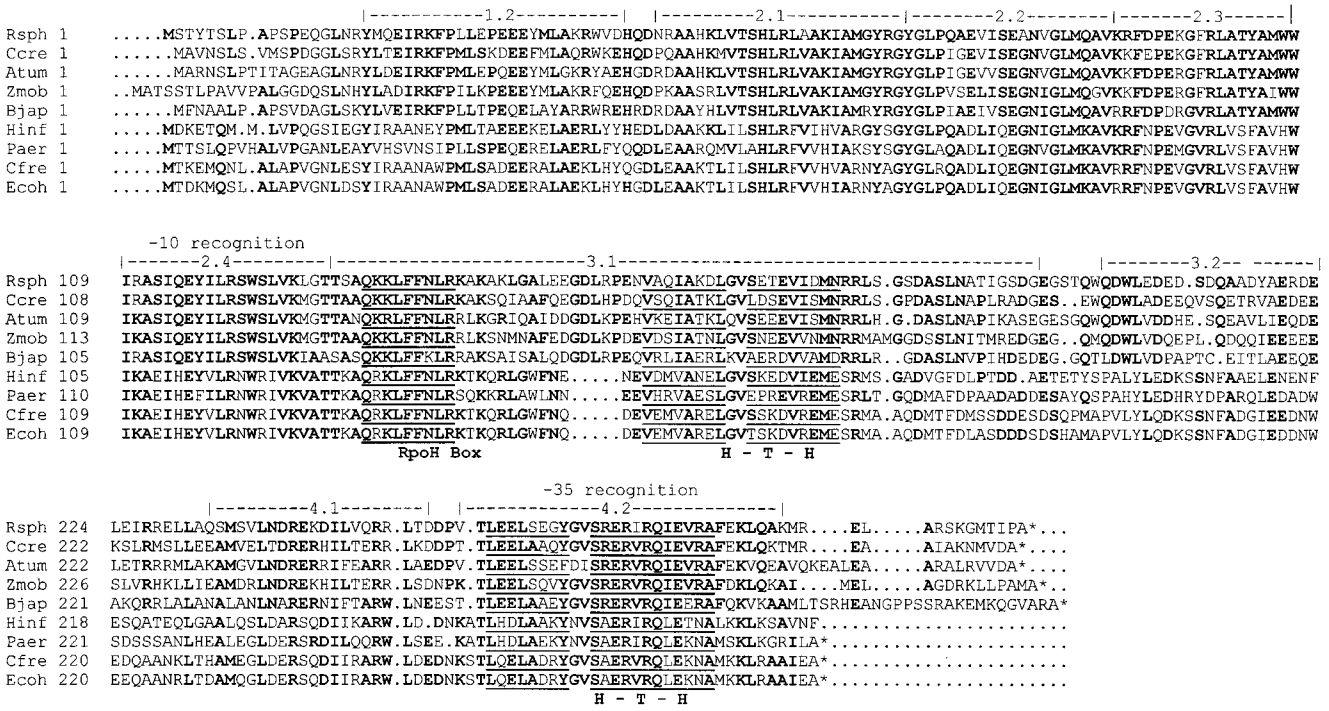


FIG. 1. Alignment of σ^{32} family members (generated by using the PILEUP program [5]). Identical amino acids are indicated in boldface. The broken lines above the alignment denote conserved regions of eubacterial sigma factors (20). GenBank accession numbers for σ^{32} family members are as follows: *C. crescentus*, U39791; *A. tumefaciens*, D50828; *Z. mobilis*, D50832; *B. japonicum*, U55047; *H. influenzae*, U32713; *P. aeruginosa*, D50052; *C. freundii*, X14960; and *E. coli*, U00039. RpoH box and helix-turn-helix (H-T-H) sequences are underlined. Gaps introduced to maximize alignment are indicated by dots. Asterisks indicate the end of the protein. Abbreviations: Rsph, *R. sphaeroides*; Ccre, *C. crescentus*; Atum, *A. tumefaciens*; Zmob, *Z. mobilis*; Bjap, *B. japonicum*; Hinf, *H. influenzae*; Paer, *P. aeruginosa*; Cfre, *C. freundii*; Ecoh, *E. coli*.

2.4 (−10 recognition) explains why Eσ^{37} transcribes *E. coli* σ^{32} promoters (16, 21; also see below). The amino acid similarity is particularly striking in a unique and highly conserved 9-amino-acid insertion [Q(R/K)KLFFNLR], designated the RpoH box (26) (Fig. 1), that has been implicated in DnaK-mediated turnover of *E. coli* σ^{32} (25). Other features conserved between *R. sphaeroides* RpoH and related proteins from nonenteric eubacteria include an insertion in region 3.1 and a C-terminal extension (Fig. 1). It appears that the known σ^{32} family members can provisionally be separated into two groups (Fig. 1), since proteins from the α proteobacteria (*R. sphaeroides*, *C. crescentus*, *A. tumefaciens*, *Zymomonas mobilis*, and *B. japonicum*) are more similar to each other and larger than their counterparts from γ proteobacteria (*E. coli*, *Haemophilus influenzae*, *Pseudomonas aeruginosa*, and *Citrobacter freundii*). The second amino acid residue in the RpoH box also seems to be a distinguishing feature; it is a lysine in proteins from the α proteobacteria and arginine in those from the γ proteobacteria.

Mapping *rpoH*. When wild-type genomic DNA was probed, *rpoH* mapped to an 1,100-kb *Ase*I restriction fragment (39; data not shown). To better position *rpoH*, we took advantage of a strain (RpoH26) containing an additional *Ase*I restriction site from the suicide plasmid pPJR29 (Table 1). When RpoH26 DNA is treated with *Ase*I, the ~1,100-kb *Ase*I restriction fragment was digested in two ~500-kb fragments (data not shown). This places *rpoH* near the center of the ~1,100-kb *Ase*I fragment or at coordinate ~2250 ± 50 kb on chromosome I.

An *R. sphaeroides* ΔRpoH strain is not temperature sensitive. To determine the role of *R. sphaeroides* σ^{37}, an *rpoH* insertion-replacement was generated in a wild-type strain. Because we expected a ΔRpoH mutant to be temperature sensitive, like its *E. coli* counterpart (44), selection for the Δ*rpoH1*::ΩSp^r allele was initially performed at temperatures between 10 and 30°C. To our surprise, cells lacking RpoH (i.e., ones that are both Sp^r and Tc sensitive) were obtained at all temperatures. To confirm the genotype of these presumptive ΔRpoH mutants, Southern blot analysis with *rpoH*, suicide plasmid (pSup202), and *spc*-specific probes was used to show that the Δ*rpoH*::ΩSp^r allele had been incorporated by an even number of crossover events (data not shown).

Respiratory phenotypes associated with the loss of *R. sphaeroides* σ^{37}. Both wild-type and ΔRpoH cells grew at temperatures up to 42°C under either aerobic conditions or when DMSO served as an anaerobic electron acceptor (Table 2). Loss of σ^{37} did not alter the ability of the lytic bacteriophage RS1 (6) to infect cells, since this virus plated at wild-type efficiency on ΔRpoH cells at all temperatures tested (Table 2). Thus, we conclude σ^{37} is not required for viability, phage replication, or energy generation in the presence or absence of oxygen.

Because resistance to toxic compounds often requires HSPs (28, 43), we compared aerobic sensitivity of wild-type and ΔRpoH cells to the heavy metal oxyanion tellurite. Wild-type cells plate at 100% efficiency at tellurite concentrations up to 300 μM, but the plating efficiency of the ΔRpoH mutant decreases by some 4 orders of magnitude between 10 and 300 μM tellurite (Fig. 2). Tellurite resistance in both strains results in oxyanion reduction, since all colonies exhibited the black phenotype diagnostic of metal deposition (23). At tellurite concentrations greater than 100 μM, plating efficiency of the ΔRpoH mutant plateaus at $\sim 10^{-3}$, suggesting that there is σ^{37}-independent pathway for heavy metal reduction. Finally, the ΔRpoH mutant appears incapable of growth at tellurite

TABLE 2. Characterization of an *R. sphaeroides* ΔRpoH mutant[a]

Growth condition and parameter	Wild type	ΔRpoH mutant
Aerobic respiration		
Temp		
30°C	+[b]	+
37°C	+	+
42°C	+	+
Tellurite	+	(see Fig. 2)
Phage growth[c]	1.0	1.3
Anaerobic respiration[d]		
Temp		
30°C	+	+
37°C	+	+
42°C	+	+
Photosynthesis		
Temp		
30°C	+	+
37°C	−	−
42°C	−	−
B875[c]	225	247
B800-850[e]	447	498
Ratio[e]	2.0	2.0

[a] Unless noted, all phenotypes were scored on solid media.
[b] +, no distinguishable difference between the behavior of wild-type and ΔRpoH cells; −, no growth.
[c] Plating efficiency of phage RS1 under aerobic conditions at 30, 37, or 42°C (6).
[d] With DMSO as an anaerobic electron acceptor.
[e] picomoles of pigment-protein complexes in $\sim 7 \times 10^8$ to 8×10^8 cells grown photosynthetically at 30°C with a light intensity of 10 W/m^2 (22). Ratio, B800-850/B875.

concentrations higher than 300 μM, since colonies were not observed when as many as 10^8 cells are plated.

Protein synthesis after aerobically grown cells are shifted to 42°C. To monitor the ability of cells to mount a heat shock response, protein synthesis was examined before and after aerobically grown cultures were placed at 42°C. In wild-type cells, a pattern of heat-inducible proteins was observed that is characteristic of that seen in other mesophilic eubacteria (14, 43). For example, the synthesis rate of several low-molecular-weight proteins (presumed Hsp10 family members) as well as those of ~65 (presumed Hsp60), 75 (presumed Hsp70), 85 (presumed Hsp90), and 120 kDa (presumed Clp family member) increased rapidly and transiently after wild-type aerobically grown cells were shifted from 30 to 42°C (Fig. 3). The response observed in wild-type cells can provisionally identify three general classes of heat-responsive proteins. The first class includes three proteins (~120, 85, and 65 kDa) whose synthesis rate increased within 5 min after a shift to 42°C and then returns to a preshift rate within 20 min (Fig. 4A, B, and D). The synthesis rates of three other proteins (~75, 17, and 14 kDa) also increased rapidly when wild-type cells were placed at 42°C, but they remained elevated even 20 min after the temperature shift (Fig. 4C, I, and J). The third class includes two proteins (~38 and 35 kDa) whose synthesis rate decreased rapidly when wild-type cells were shifted to 42°C (Fig. 4E and F).

When an aerobically grown culture of the ΔRpoH mutant was shifted to 42°C, the synthesis rates of the 38- and 35-kDa proteins were indistinguishable from those in wild-type cells (Fig. 3 and 4E and F). However, other heat-inducible proteins in wild-type cells behaved differently in the ΔRpoH mutant. For example, there was no detectable increase in the synthesis rate of the heat-inducible 120- and 85-kDa proteins when the

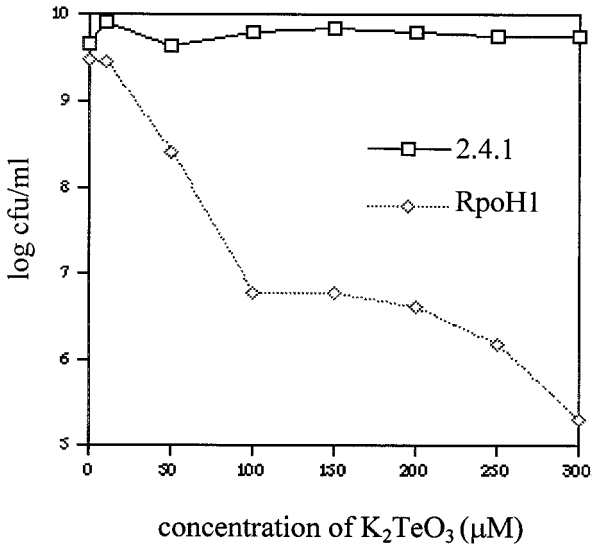


FIG. 2. Plating efficiency of aerobically grown *R. sphaeroides* strains at different tellurite concentrations. Viability of wild-type cells and the ΔRpoH mutant is expressed as the number of CFU per milliliter of original culture at each tellurite concentration.

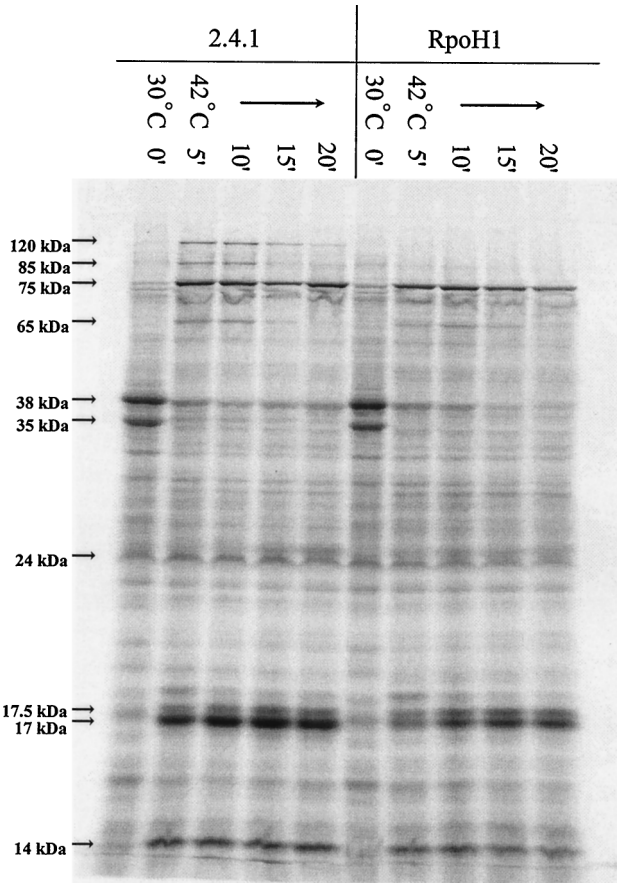


FIG. 3. Protein synthesis after aerobically grown wild-type and ΔRpoH cells are shifted from 30 to 42°C. Strains, temperatures, and sampling times (in minutes [indicated by prime symbols]) after the shift are indicated over the gel. The migration of prestained molecular mass standards (Gibco-BRL) was used to estimate the apparent molecular masses of the indicated proteins.

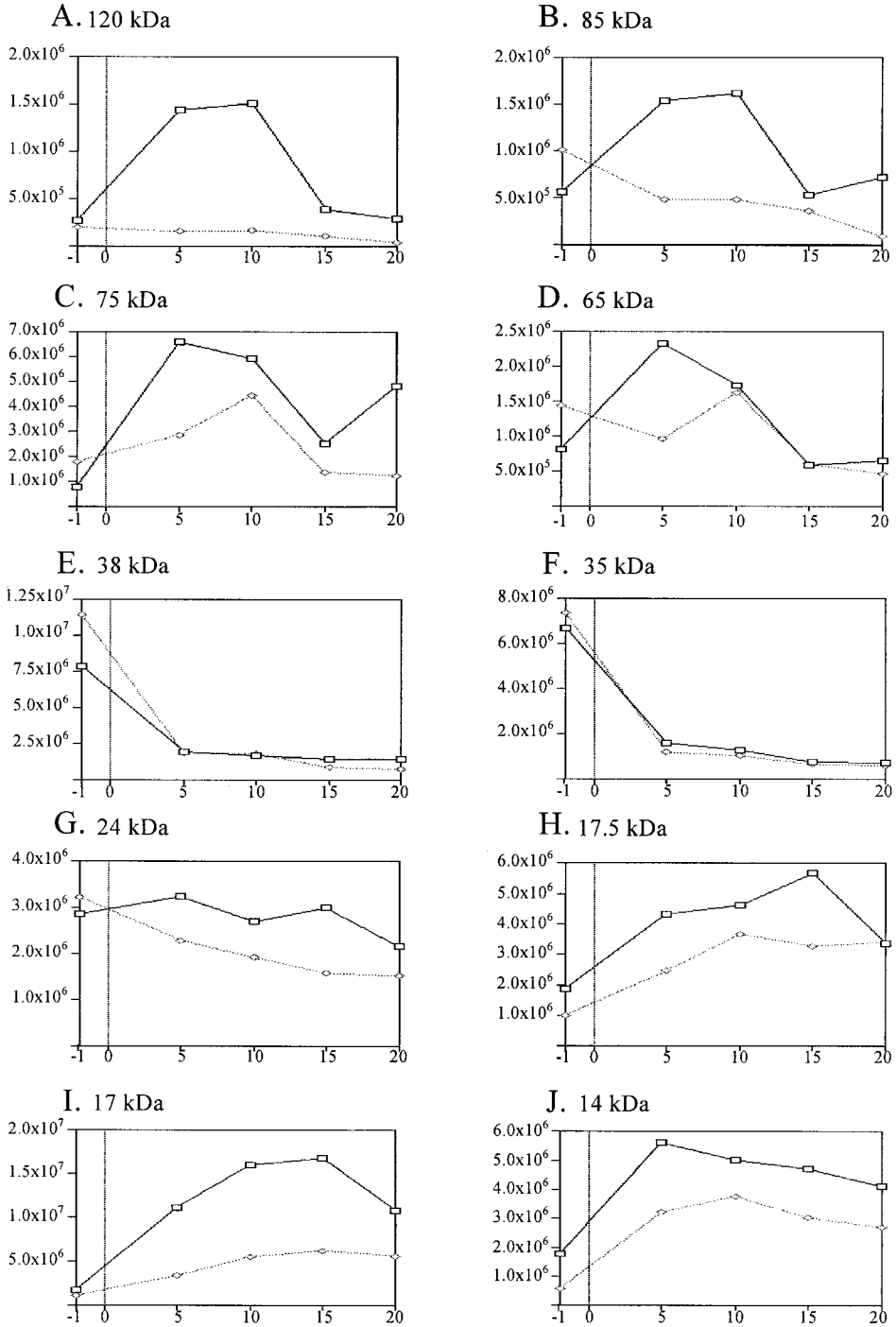
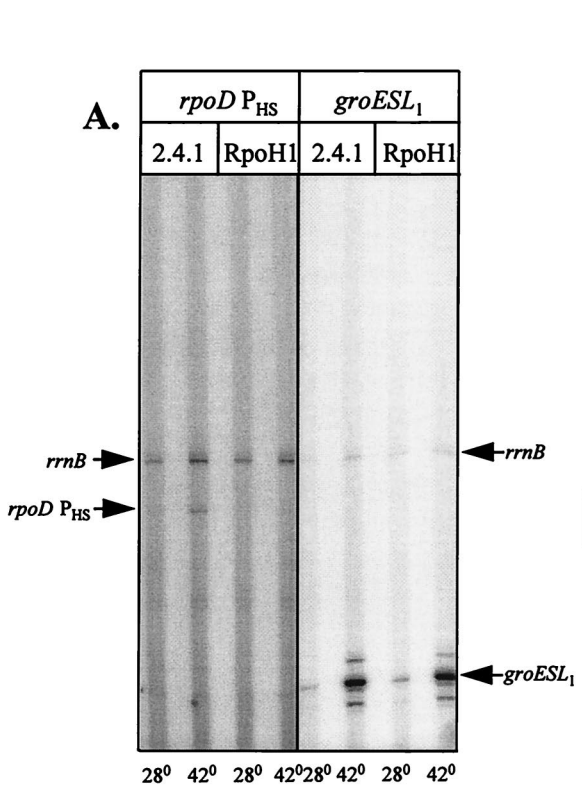
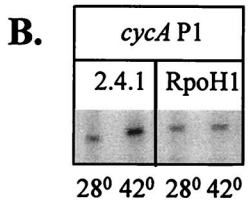


FIG. 4. Relative synthesis rates of selected proteins before and after aerobically grown cells are shifted to 42°C. The *x* axes show the minutes after exposure to 42°C (−1 indicates cells sampled 1 min before the temperature shift). The *y* axes show the relative pixel intensity of individual proteins in Fig. 5. Wild-type (□) and ΔRpoH (◇) cells are shown.

ΔRpoH mutant was placed at 42°C (Fig. 4A and B). Within 5 min after the ΔRpoH mutant was incubated at 42°C, there was also smaller increases in the synthesis rates of the heat-inducible 75- and 65-kDa proteins (Fig. 4C and D). By 20 min after the ΔRpoH mutant was placed at 42°C (Fig. 4C), the synthesis rate of the 75-kDa protein approximated that seen in cells grown at 30°C. Despite these differences, heat induction of several potential HSPs in the ΔRpoH mutant is a likely explanation for the aerobic growth of cells lacking σ^{37} at temperatures up to 42°C (Table 2).

The ΔRpoH mutant contains elevated levels of presumed heat shock transcripts after aerobically grown cells are shifted to 42°C. Primer extension assays were performed to test if loss of σ^{37} altered heat shock promoter function. For this analysis, we chose to monitor several *R. sphaeroides* promoters, *cycA* P1, *rpoH* P_{HS} and *groESL*$_1$, which have significant similarity to the





C.

promoter	2.4.1 RNA abundance 28° C	2.4.1 RNA abundance 42° C	2.4.1 fold increase	RpoH1 RNA abundance 28° C	RpoH1 RNA abundance 42° C	RpoH1 fold increase
rpoD P_{HS}	7.5×10^3	5.7×10^4	8	7.4×10^3	2.0×10^4	2.7
rrnB	6.4×10^4	1.8×10^5	2.8	7.9×10^4	1.4×10^5	1.8
groESL$_1$	1.2×10^6	2.1×10^7	17	1.8×10^6	1.9×10^7	10
rrnB	7.9×10^4	2.8×10^5	3.5	1.7×10^5	1.9×10^5	1.1
cycA P1	4.6×10^4	1.1×10^5	2.4	3.5×10^4	4.5×10^4	1.3

D.

σ^{32} consensus	TCTC-CCCTTGAAA	14 bp	CCCCAT-TA	
rpoD P_{HS}	CCCGGCCCTTGAAA	14 bp	ACCCATGTT	8 bp +1
groESL$_1$	TCCCCCCGTTGACA	13 bp	TCTCATATC	10 bp +1
rrnB	AAATCCGCTTGCGC	13 bp	CTCCTAGAA	6 bp +1
cycA P1	CCGCAGTAGTGATT	13 bp	ACCTATATC	7 bp +1

FIG. 5. Transcript levels before and 30 min after aerobically grown wild-type and ΔRpoH cells were shifted to 42°C. (A) Primer extension analysis (~8 μg of RNA per lane) of the *rrnB*, *rpoD* P_{HS}, and *groESL*$_1$ transcripts at the indicated temperatures. (B) Primer extension analysis (~8 μg of RNA per lane) of *cycA* P1-specific transcripts at the indicated temperatures. (C) Transcript abundance (pixel intensity) from panels A and B. Induction ratios denote the increase at 42°C relative to the 30°C level. (D) Comparison of potential *R. sphaeroides* promoters with the *E. coli* σ^{32} consensus sequence; matches are denoted by boldface type.

E. coli σ^{32} consensus sequence (Fig. 5D). One other, *R. sphaeroides rrnB* (10, 30), has promoter elements related to the *E. coli* σ^{70} and σ^{32} consensus sequences (Fig. 5D).

Levels of *cycA* P1, *rrnB*, *rpoD* P_{HS}, and *groESL*$_1$-specific transcripts increased ~2-, 3-, 8- and 17-fold, respectively, 30 min after aerobically grown wild-type cells were placed at 42°C (Fig. 5A to C). If the abundance of these primer extension products is taken as an estimate of promoter function, then loss of σ^{37} alters their heat inducibility because there were reproducibly smaller increases in the abundance of the *rrnB* (~1.1-fold), *cycA* P1 (~1.3-fold), *rpoD* P_{HS} (~2.7-fold), and *groESL*$_1$ (~10-fold) transcripts 30 min after the ΔRpoH mutant was placed at 42°C (Fig. 5A to C). The residual increase in *rpoD* P_{HS} and *groESL*$_1$ transcript levels after the ΔRpoH mutant was shifted to 42°C suggests that some other system is increasing promoter function when cells that lack σ^{37} are placed at a higher temperature.

Heat shock promoters are recognized by multiple *R. sphaeroides* sigma factors. To ask if recognition of heat shock promoters by another RNA polymerase holoenzyme could explain how aerobically grown ΔRpoH cells mount a heat shock response, *E. coli dnaK* P_1, *htpG*, and *rpoD* P_{HS} promoters were tested for function in vitro with *R. sphaeroides* RNA polymerase preparations. Enzyme preparations from wild-type or ΔRpoH cells (21) transcribed all of these *E. coli* heat shock promoters and produced identically sized transcripts (Fig. 6) which are indistinguishable in size to those generated by *E. coli* σ^{32} (16). The lower transcript abundance with equivalent amounts of RNA polymerase from the ΔRpoH mutant probably reflects a reduced level of the cognate holoenzyme in cells that lack σ^{37}.

To identify what *R. sphaeroides* enzyme(s) recognized these *E. coli* σ^{32} promoters, RNA polymerase holoenzymes were reconstituted by adding potential sigma factors from the ΔRpoH mutant to a core preparation (21). This analysis identified an ~38-kDa protein (σ^{38}) that allows transcription of *E. coli dnaK* P_1 when it is added to core RNA polymerase (Fig. 7). This same 38-kDa protein directs transcription of *R. sphaeroides cycA* P1 when reconstituted with core RNA polymerase subunits (21). Thus, both Eσ^{38} and Eσ^{37} recognize heat shock promoters.

Loss of σ^{37} alters the response of photosynthetic cells to increased temperature. Given the known function of HSPs like GroESL in assembly of photosynthetic functions such as ribulose-1,5-bisphosphate carboxylase (19), we asked if photosynthetic cells were particularly sensitive to the loss of σ^{37}. We were surprised to find that wild-type *R. sphaeroides* has a lower temperature maximum under photosynthetic conditions, since no growth was observed on solid media at either 37 or 42°C (Table 2). However, loss of σ^{37} does not make photosynthetic cells temperature sensitive, since the ΔRpoH strain grew normally at temperatures up to 30°C (Fig. 8 and Table 2). Photosynthetic ΔRpoH cells grown at 30°C with moderate light (22) contained levels of light-harvesting bacteriochlorophyll-protein complexes that are indistinguishable from a wild-type strain (Table 2). Thus, loss of σ^{37} does not have a significant

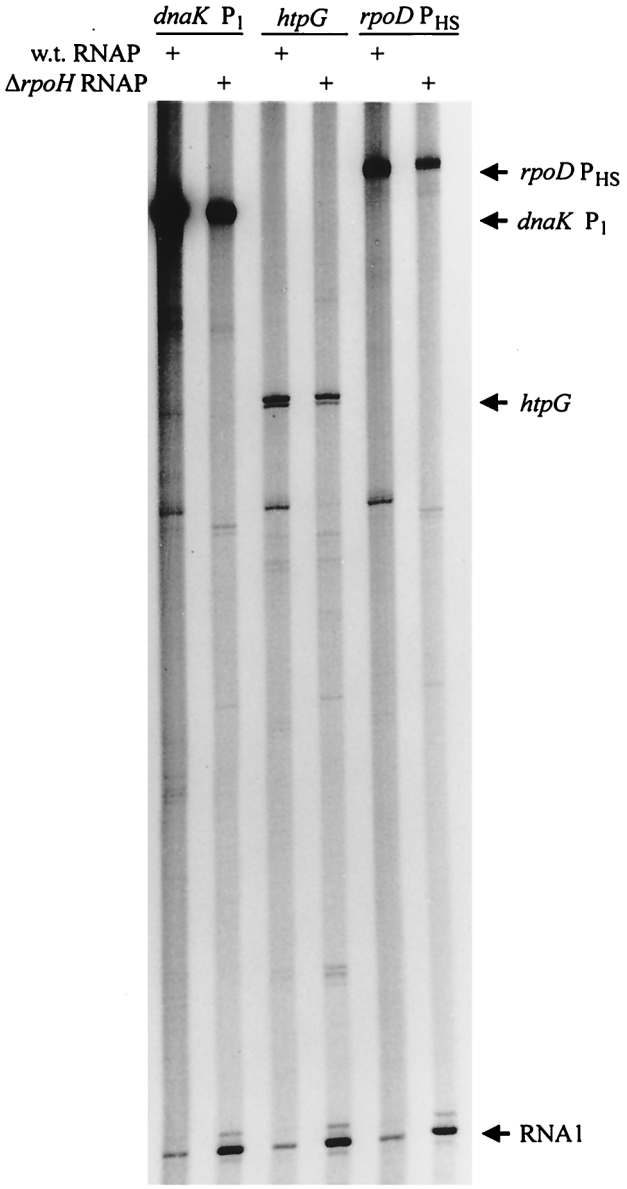


FIG. 6. Transcription of *E. coli* heat shock promoters, *dnaK* P_1, *htpG*, and *rpoD* P_{HS}, by mixtures of *R. sphaeroides* RNA polymerase holoenzymes. Wild-type (w.t.) and Δ*rpoH R. sphaeroides* RNA polymerase (RNAP) were used (+). The RNA1 transcript is a σ^{70}-dependent product from the origin of DNA replication on all templates (21).

effect on photosynthetic growth or assembly of pigment-protein complexes at 30°C.

During these experiments, we noted a difference in the response of wild-type and ΔRpoH cells to increased temperature under photosynthetic conditions. When a wild-type photosynthetic culture was shifted from 30 to 37°C, there was typically an ~1.5- to 2.5-fold increase in culture turbidity after the temperature increase (Fig. 8). These turbidity increases cease long before those in a control culture maintained at 30°C. Microscopic examination of wild-type photosynthetic cultures several hours after the shift to 37°C revealed a significant number of paired cells or doublets in the population (data not shown). One explanation for this behavior is that wild-type photosynthetic cultures only complete existing rounds of DNA replication or cell division after the temperature is elevated. Unfortunately, exposure to 37°C under photosynthetic conditions is bactericidal (data not shown), so we were unable to observe increases in CFU after photosynthetic cells were placed at 37°C. In the ΔRpoH mutant, increases in culture turbidity cease rapidly when photosynthetic cultures are shifted from 30 to 37°C (Fig. 8). There are no microscopic indications for the formation of cell doublets after photosynthetic cultures of the ΔRpoH mutant were placed at 37°C (data not shown). Compared with wild-type cells, photosynthetic cells lacking σ^{37} seem unable to complete previously initiated cell division cycles after exposure to 37°C.

To gain additional insight into potential reasons for this behavior, we monitored bulk protein synthesis before and after photosynthetic cells were shifted from 30 to 37°C. At 30°C, there are significant differences between the protein synthesis pattern of photosynthetic and aerobically grown cultures (compare Fig. 3 to Fig. 9), but the protein synthesis patterns of wild-type and ΔRpoH cells are indistinguishable both before and after photosynthetic cells were incubated at 37°C (Fig. 9). Thus, the different growth response when photosynthetic cultures are shifted to 37°C (Fig. 8) cannot reflect a cessation of translation after the temperature increase. Of equal significance, no detectable heat shock response was observed when photosynthetic wild-type or ΔRpoH cells were shifted to 37°C (compare Fig. 3 and 9); only proteins of ~62 and 17 kDa exhibited a significant increase in their synthesis rate when photosynthetic cultures of either strain were shifted to 37°C (Fig. 9). Thus, the failure to mount a heat shock response at 37°C under photosynthetic conditions is a likely explanation for why wild-type and ΔRpoH cells are unable to grow at this temperature.

DISCUSSION

It was previously suggested that *R. sphaeroides* $E\sigma^{37}$ recognized *E. coli* heat shock promoters because an RNA polymerase fraction containing a 37-kDa protein transcribed these genes in vitro (16). The amino acid similarity between *R. sphaeroides* RpoH and proteins in the eubacterial heat shock sigma factor family, its ability to restore bacteriophage λ sensitivity to an *E. coli* σ^{32} null mutant, and the heat shock gene expression seen when σ^{37} is expressed in *E. coli* cells lacking σ^{32} indicate that $E\sigma^{37}$ recognizes promoters related to the *E. coli* σ^{32} consensus sequence. The observation that RNA polymerase preparations from an *R. sphaeroides* ΔRpoH mutant lack this 37-kDa protein further suggests that *rpoH* encodes σ^{37} (21). Additional conclusions and questions generated by this characterization of *R. sphaeroides* RpoH are presented below.

***R. sphaeroides* RpoH is essential only under limited conditions.** Growth of cells lacking σ^{37} at the same temperatures as their wild-type counterparts was surprising when one considers that *E. coli* ΔRpoH mutants cannot grow at temperatures above 20°C (44). The ability of *R. sphaeroides* ΔRpoH cells to support infection by the lytic RS1 virus at all temperatures tested also contrasts with the inability of *E. coli* ΔRpoH mutants to propagate phages such as λ (which served as the basis for our isolation of *R. sphaeroides rpoH*). Indeed, only two conditions were found where the *R. sphaeroides* ΔRpoH mutant had a phenotype.

One difference between wild-type and ΔRpoH cells was the increased aerobic sensitivity of cells lacking σ^{37} to the toxic heavy metal oxyanion tellurite (23). Increased sensitivity of the *R. sphaeroides* ΔRpoH mutant to tellurite could reflect a limitation of HSPs or other members of a presumed σ^{37} regulon. While an *R. sphaeroides* RdxA mutant has a tellurite-sensitive

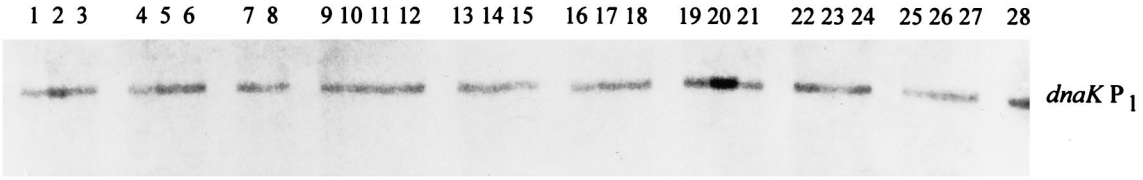


FIG. 7. *R. sphaeroides* Eσ^{38} recognizes *E. coli dnaK* P_1. RNA polymerase holoenzymes were reconstituted by adding potential sigma factors (lanes 1 to 28) from the ΔRpoH mutant to a core RNA polymerase preparation from the ΔRpoH mutant (21). The protein in lane 20 was the 38-kDa polypeptide (σ^{38}) that allowed core RNA polymerase to transcribe the *cycA* P1 heat shock promoter (21).

phenotype reminiscent of the ΔRpoH mutant (29), it is not known if this or proteins directly involved in heavy metal reduction are transcribed by Eσ^{37}. Alternatively, one promoter for the cytochrome c_2 gene (*cycA* P1) is transcribed by *R. sphaeroides* Eσ^{37} (21). If cytochrome c_2 transferred electrons to the membrane-bound reductase implicated in tellurite reduction (23), then increased *cycA* P1 activity might be required for heavy metal resistance. This might also explain why tellurite reduction was blocked in cytochrome c_2 null mutants (23).

Another phenotype associated with loss of *R. sphaeroides* σ^{37} was the rapid cessation in culture turbidity increases when photosynthetic cells were shifted from 30 to 37°C. This behavior of the ΔRpoH mutant does not reflect a total block in macromolecular synthesis, since translation continues after photosynthetic cells are shifted to 37°C. However, the failure of both the ΔRpoH mutant and wild-type cells to mount a heat shock response at 37°C under photosynthetic conditions suggests they are limited for the HSPs needed to progress through the cell cycle. If a component of the photosynthetic apparatus were temperature sensitive, this behavior could reflect a lack of energy for HSP function after such cells are placed at 37°C.

***R. sphaeroides* mounts a heat shock response in the presence and absence of σ^{37}.** From the universal nature of the heat shock response, it is not surprising that synthesis rates of a number of proteins and the transcript levels from several potential heat shock genes increase after aerobic cells are placed at 42°C (42). Heat induction of several proteins was transient, since the synthesis rate of several putative HSPs (Clp, Hsp90, and Hsp60 homologs) decreased to a new steady state within 20 min after temperature up shift. Other potential HSPs (Hsp70 homologs and several lower-molecular-weight polypeptides) continued to be synthesized at an increased rate even after 20 min at 42°C. Such a persistent induction of HSPs is not seen in well-studied systems like *E. coli* (14, 43).

When the aerobic heat shock response is analyzed, induction of ~75-, 65-, and 17-kDa proteins seems partially dependent on σ^{37}, since the magnitude or timing of their synthesis is altered when the ΔRpoH mutant is shifted to 42°C. If the heat-inducible ~65-kDa protein is a product of the *R. sphaeroides groESL*$_1$ operon (19), then this reduction probably reflects altered promoter function in cells lacking σ^{37}, since we observed a diminished increase in this transcript 30 min after the ΔRpoH mutant was placed at 42°C. Heat induction of another group of presumed HSPs (~120 and 85 kDa) could be totally dependent on σ^{37}, since their rate of synthesis is not measurably increased in the ΔRpoH mutant.

The temperature maxima of *R. sphaeroides* varies with its mode of energy generation. We were surprised by the selective inability of *R. sphaeroides* to grow or mount a heat shock response under photosynthetic conditions at temperatures ≥37°C. Photosynthetic temperature maxima of ~35°C have been reported for many wild-type *R. sphaeroides* strains (9), yet we have shown that strain 2.4.1 grows and mounts a heat shock response at 42°C when it is generating energy by aerobic or anaerobic respiration. This conditional difference in temperature profile does not reflect an inability to synthesize photosynthetic pigments, since cells using DMSO as an anaerobic electron acceptor at 42°C have colony pigmentation characteristic of the presence of bacteriochlorophyll-protein complexes (data not shown).

***R. sphaeroides* has two sigma factors which recognize heat shock promoters.** One likely reason why cells lacking σ^{37} mount a heat shock response is a 38-kDa protein (σ^{38}) that directs core RNA polymerase to recognize σ^{32} promoters like *E. coli dnaK* P_1 and *R. sphaeroides cycA* P1 (21). A similar duplication of RpoH function exists in *B. japonicum* where a second related gene is evident in genomic Southern blots probed with *E. coli rpoH* (27). At the stringency conditions we employed, no additional sequences related to *R. sphaeroides rpoH* were observed in genomic Southern blots (data not shown). Until information is available on σ^{38}, its sequence and

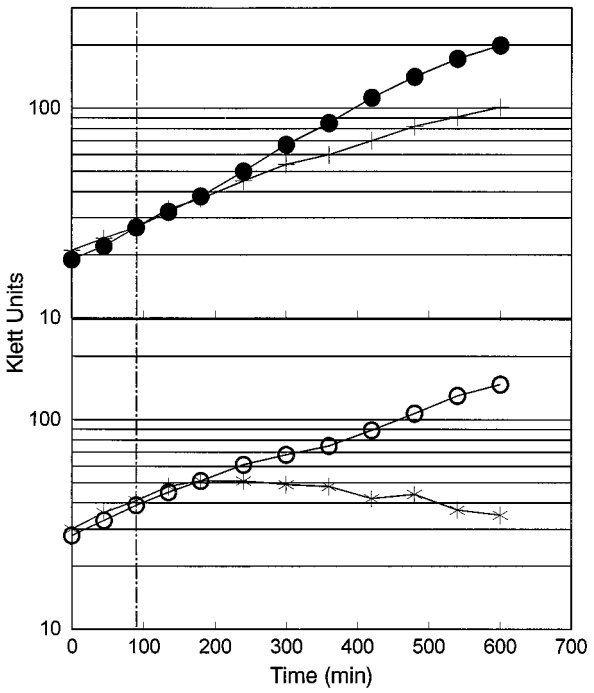


FIG. 8. Photosynthetic growth of wild-type cells (top) and the ΔRpoH mutant (bottom) at 30°C and after a temperature shift from 30 to 37°C. Photosynthetic growth of wild-type cells (●) and those lacking σ^{37} (○) at 30°C and the response seen when wild-type cells (+) or the ΔRpoH (✳) mutant are shifted from 30 to 37°C are shown. The broken vertical line indicates when the photosynthetic cultures of wild-type and ΔRpoH cells were placed at 37°C.

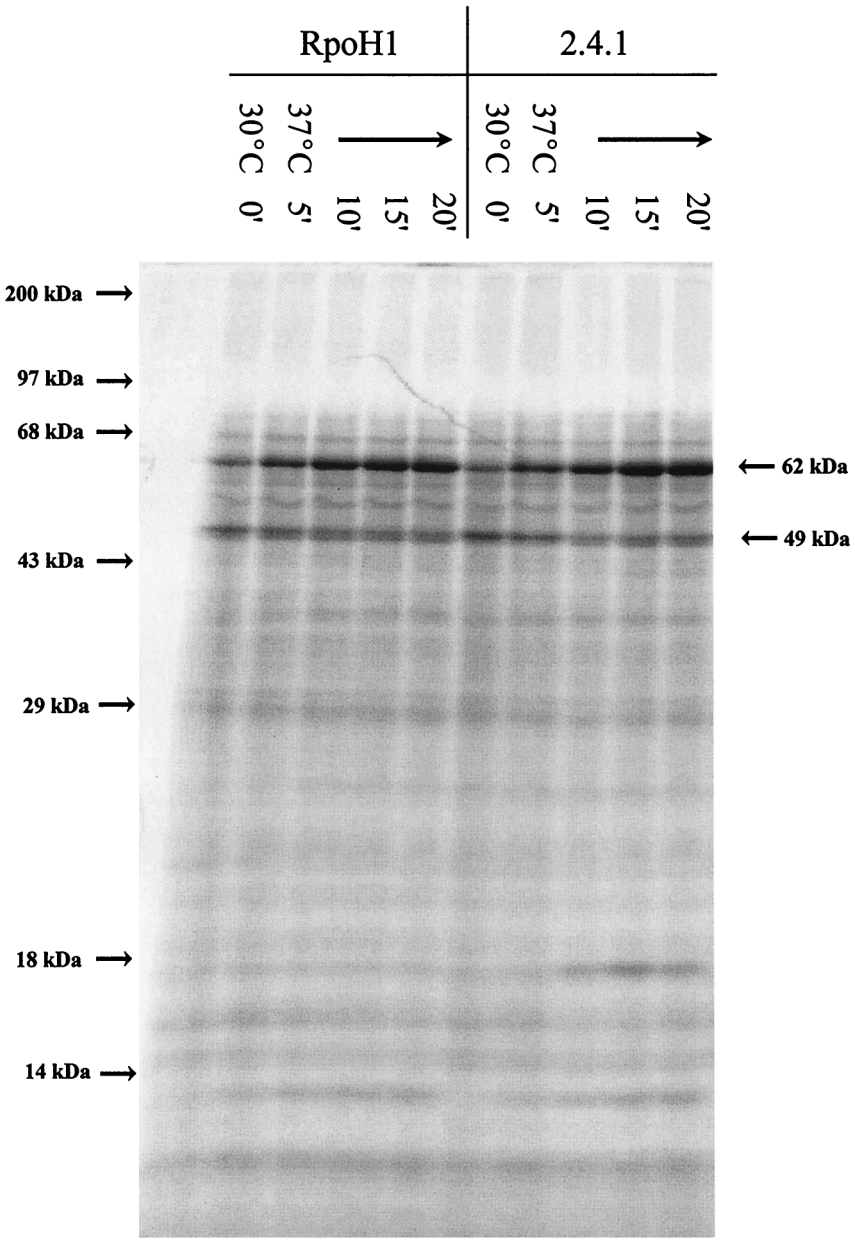


FIG. 9. Protein synthesis before and after photosynthetic wild-type and ΔRpoH cells are shifted from 30 to 37°C. Temperatures and sampling times (in minutes [indicated by prime symbols]) after the shift are indicated over the gel. The migration of molecular mass standards (Gibco-BRL) indicated to the left of the gel were used to estimate the apparent sizes of the indicated proteins.

functional similarity to members of the σ^{32} family remain open questions.

A *B. japonicum* Δ*rpoH* mutation also does not cause temperature sensitivity, presumably because this α proteobacterium contains other related sigma factors (27). Thus, it will be interesting to see if the existence of a second sigma factor that transcribes heat shock genes extends to the other eubacteria from which proteins in the σ^{32} family have been identified.

The presence of a CIRCE element in the *R. sphaeroides* $groESL_1$ operon (19) suggests that other mechanisms can contribute to increased heat shock gene expression. The discovery that mutations which increase activity of an *R. sphaeroides* σ^E homolog alters expression of several genes (30a, 34, 35), including one recognized by σ^{37} (21), reveals additional potential components of this bacterium's response to environmental or metabolic stress. Experiments are in progress to define the roles and metabolic signals for these alternative *R. sphaeroides* sigma factors.

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