Proc. Natl. Acad. Sci. USA
Vol. 86, pp. 5010–5014, July 1989
Genetics

An *apaH* mutation causes AppppA to accumulate and affects motility and catabolite repression in *Escherichia coli*

(oxidative stress/σ factor/dinucleotides)

SPENCER B. FARR, DAVID N. ARNOSTI, MICHAEL J. CHAMBERLIN, AND BRUCE N. AMES†

Department of Biochemistry, University of California, Berkeley, CA 94720

Contributed by Bruce N. Ames, February 15, 1989

ABSTRACT ***apaH*$^-$ mutants lack the hydrolase responsible for degradation of AppppN dinucleotides in *Escherichia coli* and show a ≥16-fold increase in AppppA under nonstress conditions. These mutants lack detectable activity of σ^F, a factor required for transcription of motility and chemotaxis genes. Expression of the *flbB/flaI* operon, thought to encode σ^F, is decreased in *apaH*$^-$ mutants, and there appears to be a general decrease in expression of genes regulated by cAMP-binding protein and cAMP as well.**

The dinucleotide P^1,P^4-bis(5′-adenosyl) tetraphosphate (AppppA; diadenosine tetraphosphate) is formed by aminoacyl-tRNA synthetases in a reaction in which an enzyme-bound aminoacyladenylate intermediate donates AMP to ATP (1, 2). The cellular concentration of adenylylated nucleotides, including AppppA, rises rapidly when the cell is exposed to heat shock or oxidative stress (3–5). It has been postulated that various nucleotide derivatives serve as cellular signals ("alarmones") of stress conditions (3, 4, 6). The best understood cases of small phosphorylated molecules acting as stress signals, or alarmones, are ppGpp and cAMP; the former signals amino acid starvation and the latter signals an insufficient source of fuel or carbon (7, 8). In support of the hypothesis that dinucleotides serve as alarmones, Kramer *et al.* (9) have shown that *nuv* mutants in *Salmonella typhimurium*, which cannot produce ApppGpp when exposed to near-UV light, fail to produce several proteins made in the wild type and are sensitive to killing by near-UV light.

The role of AppppA in oxidative or heat shock responses has not been determined, though it has been shown that AppppA inhibits the 5′-nucleotidase activity of the heat shock protein DnaK *in vitro* (10). The *lysU* gene, which encodes one of two lysyl-tRNA synthetases in *Escherichia coli*, produces much of the AppppA in *E. coli* (11). *lysU* is a heat-shock-inducible gene (5), which suggests that AppppA levels may indeed play a role in the cell's response to heat stress.

There also exists evidence against AppppA serving as the alarmone for heat shock or oxidative stress. In *E. coli*, *in vitro* hydrolysis of AppppN is attributed to a single enzyme, P^1,P^4-bis(5′-adenosyl) tetraphosphate pyrophosphohydrolase (12). By using a high-copy-number plasmid carrying the gene (*apaH*) that encodes this hydrolase (13), it has been shown that heat shock protein production was normal even when AppppA levels were dramatically reduced (12). It has also been shown that AppppA synthesis lags behind heat shock protein synthesis (5). These results suggest that AppppA is not the signal to induce the heat shock response.

To determine the role AppppA plays in moderating cellular responses to stress in *E. coli*, we constructed an *apaH*$^-$ mutant. This mutant shows a ≥16-fold increase in the basal levels of AppppA at 30°C. In this report we show that an *apaH*$^-$ mutation causes decreased transcription of motility and chemotaxis genes and that it does so by inhibiting the transcription of chemotactic regulatory operon *flbB/flaI*. *flbB/flaI* is itself regulated by the complex of cAMP-binding protein (CAP) and cAMP, and we show that its expression as well as expression of many other CAP–cAMP-regulated genes is diminished in *apaH*$^-$ mutants.

MATERIALS AND METHODS

Strain Construction. Strains used are listed in Table 1. Matings and phage P1 transductions (ref. 19, pp. 201–204 and 190–195), transformations (20), Tn::mini-*kan* insertion mutagenesis (21), and Southern analysis (22) were done as described.

Two-Dimensional (2-D) Thin-Layer Chromatography. Cells were grown overnight in Mops medium with 0.4% glucose and thiamin, histidine, arginine, proline, leucine, and threonine at 10 μg/ml each. *apaH*::Tn*kan* strains were grown in kanamycin sulfate at 40 μg/ml. Overnight cultures were diluted 6-fold in the same medium and 1.2 μmol of $^{32}PO_4$ (150 μCi/μmol (Amersham; 1 Ci = 37 GBq) was added. Extracts were then prepared and chromatographed as described (3). Twenty microliters of each extract was spotted onto each plate as well as 5 μl of 1 mM unlabeled AppppA to facilitate identification of ^{32}P-labeled AppppA.

We quantified AppppA as described previously (23) with the following modifications for *E. coli* cells. Cells were grown in LB medium (ref. 19, p. 433) at 30°C (plus kanamycin sulfate at 40 μg/μl for *apaH*::Tn*kan* mutants) to OD_{600} of 0.3–0.4. Cells from 80 ml of culture were harvested by filtration and lysed in 8 ml of 9% (wt/vol) formic acid.

ATP, Galactokinase, and β-Galactosidase Assays. ATP levels were measured as described (24) with the following modifications. Five hundred microliters of assay mixture was added to 250 μl of cold 24% (wt/vol) perchloric acid. After 20 min on ice, samples were centrifuged and 500 μl of supernatant was neutralized with 125 μl of 4 M KOH and 125 μl of 2 M $KHCO_3$. After 30 min on ice, samples were centrifuged again and the supernatant was assayed for ATP. β-Galactosidase (ref. 19, pp. 403–404) and galactokinase assays were as described (25) but cells were grown in 0.3% unlabeled galactose for the galactokinase assay.

Analysis of *tar* Transcripts Produced *in Vivo*. RNA was isolated from 20-ml cultures of AB1157 (*apaH*$^+$) with or without pDNA1 and SF436 (*apaH*$^-$) with or without pDNA1 in LB medium containing antibiotics where appropriate to OD_{600} = 0.4–0.5 as described (26). Total RNA (30 μg) was analyzed by using a *tar* transcript-specific oligonucleotide in primer extension reactions as described (26).

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Abbreviations: CAP, cAMP-binding protein; 2-D, two-dimensional; Km^R, kanamycin resistance.

†To whom reprint requests should be addressed.

Table 1. List of strains

Name	Relevant genotype	Source or ref.
E. coli		
AB1157	*argE3 hisG-4 leu-6 proA2 thr-1 arg-14 galK2 lacY1 mtl-1 xyl-5 tsx-33 sup-37 str-31*	14
SF436	As AB1157 but *apaH*::Tn*kan*	This work
SF495	As SF436 but F'104	15
MS4136	*flaI araD139* Δ*lac*(u169) *strA thi pyrC nalA thyA his*	16
YK3421	*hag*::Mud1 *araD139* Δ*lac*(u169) *strA thi pyrC46 nalA thyA his*	16
SF491	As YK3421 but *apaH*::Tn*kan*	This work
YK4323	*flaI*::Mud1 *araD139* Δ*lac*(u169) *strA thi pyrC46 nalA thyA his*	16
SF492	As YK4323 but *apaH*::Tn*kan*	This work
SF43	*trp thyA ilv proB metE deoC*	
SF506	As SF43 but *apaH*::Tn*kan*	This work
JC7623	*recB recC sbcB*	17
Plasmid		
pAP47	*apaH*	13
pPM61	*flbB flaI*	18
pDNA1	Truncated *tar* gene	This work
pSF32	As pAP47 but *apaH*::Tn*kan*	This work

F'104 transfers genes from *thr* through *argF*.

***In Vitro* Transcription of *tar* by Crude Extracts.** Cells were grown at 30°C in 1 liter of LB medium to OD_{600} = 1.0, collected by centrifugation, and immediately frozen in a dry ice/ethanol bath. Cell pellets were weighed, mixed with twice their weight of levigated alumina, and ground with mortar and pestle for 10 min at 4°C. Next, 1 ml of lysis buffer [10 mM Tris·HCl, pH 8.0/10 mM $MgCl_2$/1 mM EDTA/50 mM NaCl/7.5% (vol/vol) glycerol/30 μM dithiothreitol/290 μM phenylmethanesulfonyl fluoride) was added per g of alumina. The mixture was centrifuged at 3000 × *g* for 5 min, then the supernatant was transferred to a new tube and recentrifuged at 27,000 × *g* for 30 min. The protein concentration in the resulting supernatant was measured by using the Bradford assay (Bio-Rad). *In vitro* transcriptions were performed by adding crude extract and lysis buffer (10 μl total volume) to 40 μl of transcription buffer, giving final concentrations of 40 mM Tris·HCl at pH 8.0, 10 mM $MgCl_2$, 10 mM 2-mercaptoethanol, acetylated bovine serum albumin at 0.1 mg/ml, pDNA1 plasmid DNA at 40 μg/ml, and 1 mM each ATP, UTP, CTP, and GTP, and incubating for 60 sec at 30°C. The reaction was stopped and extracted by addition of 50 μl each of phenol, chloroform/isoamyl alcohol (24:1, vol/vol), and 10 mM Tris·HCl, pH 8.0/1 mM EDTA. The reaction mixtures were re-extracted with 100 μl of chloroform/isoamyl alcohol and RNA was precipitated with 250 μl of ethanol. mRNA was analyzed by primer extension as described (26).

RESULTS

Constructing an *apaH*::Tn*kan* Insertion Mutation. A λ NK1105 (21) carrying a Tn::mini-*kan* minihop element was used (21) to insert a 1840-base-pair (bp) fragment of DNA encoding kanamycin resistance (Km^R) into the center of the *apaH* gene carried on plasmid pAP47 (13). The position and orientation of the Tn::mini-*kan* minihop insert were determined by restriction mapping and Southern analysis (data not shown). A 4.9-kilobase (kb) *Eco*RI–*Sph* I linear fragment carrying the *apaH*::Tn*kan* fusion flanked on either side by ≥0.6 kb of genomic DNA was isolated and used (27) to transform a *recB recC sbcB* strain (27). A P1 lysate was made on the *recB recC sbcB apaH*::Tn*kan* transformant and was used to transduce AB1157 to Km^R. That the $apaH^+$ allele had been replaced with the *apaH*::Tn*kan* insert was confirmed by P1 mapping. The *leu* operon is slightly less than 1 min away from the *apaH* locus (28). The Km^R insert cotransduced 45.6% with *leuB* (73 out of 160), thus strongly suggesting that the *apaH*::Tn*kan* insert mapped to the correct position. To further confirm that the *apaH* allele had been replaced by the *apaH*::Tn*kan* insert, genomic DNA from wild type and the putative *apaH*::Tn*kan* strain was isolated, digested with *Eco*RI and *Xho* I, blotted, and probed with an *Eco*RI–*Acc* I fragment from pAP47 that spans the *apaH* gene. The 1840-bp Km^R insert has one *Xho* I site, whereas there are no *Xho* I sites between the *Eco*RI and *Acc* I sites on the wild-type *apaH* locus. The wild-type strain showed only one band that hybridized to the probe, while the mutant showed two bands, thus confirming that the $apaH^+$ gene had been replaced by the *apaH*::Tn*kan* insertion (data not shown).

Dinucleotide Levels Are Increased in the *apaH* Mutant. As *apaH* encodes the major AppppN hydrolase, it was expected that AppppN levels would be higher in the mutant than in the wild type. To determine which dinucleotides showed altered levels, 2-D thin-layer chromatography (3) was performed on cell extracts from wild type and $apaH^-$ mutants. Fig. 1 shows that AppppA is the only dinucleotide elevated in the $apaH^-$ mutant.

The increase in AppppA in the $apaH^-$ mutant was also measured by using a chemiluminescence assay (12). When cells were grown in LB medium at 30°C to OD_{600} of 0.2–0.3, AppppA concentrations were 16-fold higher in the $apaH^-$ mutant than in the wild type, 16 μM and 1 μM, respectively. At OD_{600} of 1.0, the AppppA in the mutant rose to 100 μM while remaining unchanged in the wild type. ATP concen-

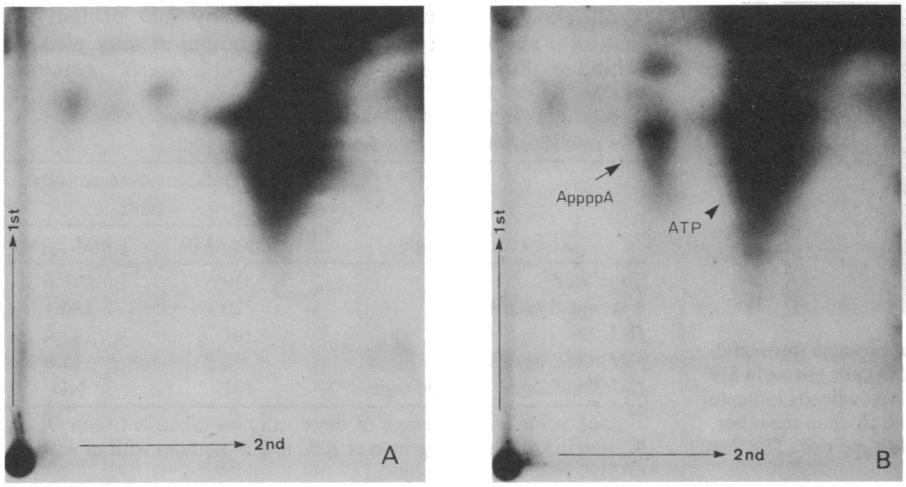

FIG. 1. Nucleotides from $apaH^+$ (AB1157) and $apaH^-$ (SF436) cells (*A* and *B*, respectively). Autoradiograms were exposed from 2-D thin-layer chromatograms of ^{32}P-labeled extracts of cells prepared as described in *Materials and Methods*. AppppA and ATP spots are labeled. A control autoradiogram of nucleotides from an $apaH^-$/F'$apaH^+$ merodiploid grown under the same conditions was identical to the wild type and is therefore not shown.

trations were measured (24), and no significant difference was found between the wild type and *apaH*$^-$ mutants (1.0 mM and 1.1 mM, respectively). An F′ episome carrying a wild-type *apaH* gene was mated into the *apaH*$^-$ strain (SF495), and it showed an AppppA concentration similar to that in wild-type cells (1.8 μM at OD_{600} = 0.3).

2-D Gel Analysis of *apaH* Mutants. To determine if an increase in the constitutive level of AppppA affected the protein synthesis of the host, 2-D gel electrophoresis was performed on wild type and *apaH*$^-$ mutants. Cells were grown at 30°C and labeled 7 min with [^{35}S]methionine, and crude lysate was electrophoresed (29). Fig. 2 shows that there are at least 14 proteins whose synthesis is decreased in the *apaH*$^-$ mutant compared to the wild type. These include flagellin, the chemotaxis receptors Tar and Tsr (30), and the heat shock protein GroEL. Increased AppppA did not result in the induction of other heat shock proteins or the synthesis of OxyR-regulated proteins. *apaH*$^-$ mutants also show increased synthesis of 7 unidentified proteins compared to the wild type.

***apaH*$^-$ Mutants Are Nonmotile.** One of the phenotypes associated with an *apaH*$^-$ mutation is that cells are nonmotile. The loss of motility in *apaH*$^-$ mutants was scored by inoculating cells into swarm stabs and swarm plates, and by observation under a microscope (Fig. 3). This finding is consistent with the 2-D protein gels, which show an absence of flagellin (Hag protein; Fig. 2).

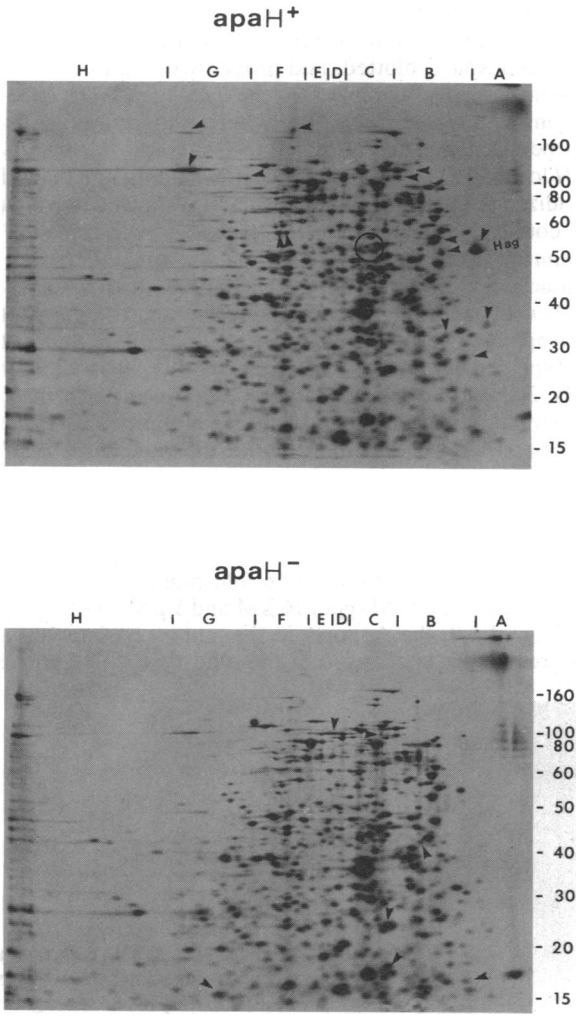

FIG. 2. Two-dimensional autoradiograms of proteins from wild-type *apaH*$^+$ (AB1157) and *apaH*$^-$ mutant (SF436) cells grown in M9 salts plus glucose and Casamino acids at 30°C. Arrowheads indicate proteins that are in higher concentration in one strain than the other. The flagellin protein (Hag) is labeled in the wild-type cell. The Tar and Tsr proteins are circled.

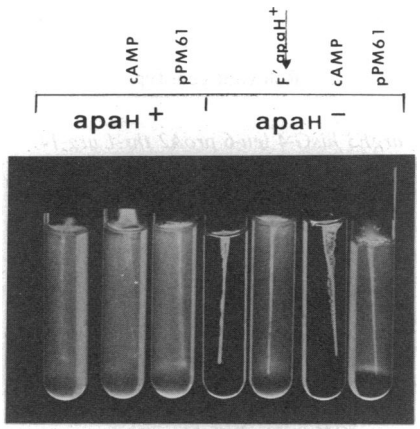

FIG. 3. Motility in wild-type and *apaH*$^-$ cells. LB/0.35% agar tubes were stab inoculated from a single colony and incubated 36 hr at 30°C. Prior to pouring tubes, kanamycin was added to 40 μg/ml to all tubes inoculated with SF436 derivatives. Ampicillin was added to 50 μg/ml to tubes inoculated with strains carrying pPM61, and cAMP was added to 1 mM to indicated tubes. Turbidity outside the stab indicates motility.

Flagella formation, and therefore motility, has been shown to require cAMP (31, 32). While cAMP was undetected in either wild-type or *apaH*$^-$ extracts by 2-D thin-layer chromatography, it remained of interest to determine if *apaH*$^-$ mutants were nonmotile due to decreased cAMP. Motility was assayed with and without the addition of exogenous cAMP. cAMP (1 mM) had no effect upon *apaH*$^-$, showing that lack of motility in *apaH*$^-$ mutants is not a function of decreased cAMP levels (Fig. 3). The *apaH*$^-$ mutant carrying an F′ episome (*apaH*$^+$) showed normal motility, indicating that the *apaH*$^-$ mutation caused the loss of motility in the mutant.

Transcription of the Flagellin Gene (*hag*) Is Decreased in *apaH* Mutants. Protein gels revealed that flagellin synthesis was undetectable in the *apaH*$^-$ mutant. To determine if decreased flagellin was due to decreased transcription of the *hag* gene, β-galactosidase levels were measured in *apaH*$^+$ and *apaH*$^-$ *hag*::*lacZ* fusion strains. Table 2 shows that transcription of *hag* is significantly decreased in the *apaH*$^-$ strain. Synthesis of flagellin is partially controlled by cAMP levels (31). Addition of cAMP slightly stimulated expression of *hag* expression in both *apaH*$^+$ and *apaH*$^-$ backgrounds. The cAMP enhancement is small, since in LB medium without glucose, expression of motility and chemotaxis genes is already induced (for example, see Fig. 3). While *hag* gene transcription is decreased by approximately 70%, flagellin synthesis is completely undetectable by 2-D protein gel analysis, suggesting that flagellin production may also be inhibited at the post-transcriptional level in *apaH*$^-$ mutants.

Table 2. Effect of increased AppppA on *hag* and *flaI* transcription

Relevant genotype	β-Galactosidase activity, units	
	No cAMP	1 mM cAMP
hag::*lacZ*	319	413.6
hag::*lacZ apaH*$^-$	120	156
flaI::*lac*	107	142.4
flaI::*lacZ apaH*$^-$	12.2	15.8
flaI::*lacZ apaH*$^-$ F′104 (*apaH*$^+$)	101	ND

Each value is the average of three samples taken between OD_{600} 0.2 and 0.3. Cells were grown at 30°C in LB medium with or without cAMP. ND, not determined.

An *apaH* Mutation Decreases Expression of σ^F-Regulated Genes. Genes encoding flagellin (*hag*), the aspartate receptor (*tar*), and other motility and chemotaxis proteins in *E. coli* share sequence homology in their promoter regions (18, 33), are coordinately regulated (16), and are transcribed by an RNA polymerase containing an alternative σ, σ^F (26). Since *apaH*$^-$ cells synthesized less flagellin, Tar, and Tsr proteins, and since flagellin synthesis was altered at the level of transcription, it was of interest to determine if other σ^F-regulated genes showed decreased transcription. To test this, RNA was isolated from wild type and *apaH*$^-$ mutants carrying pDNA1, a plasmid encoding part of the *tar* gene (26). The *tar* promoter is not utilized by σ^{70} holoenzyme (26). Primer extension analysis was used to detect 5′ ends of transcripts generated *in vivo* from the plasmid-encoded *tar* gene. Fig. 4 shows that while *apaH*$^+$ cells transcribe the *tar* gene carried on the plasmid, *apaH*$^-$ mutants do not. Transcripts arising from the chromosomal copy of the *tar* gene were apparently not abundant enough to detect. These results show that down-regulation of σ^F-controlled genes in the *apaH*$^-$ mutant occurs, at least in part, at the level of transcription.

Crude Extracts of *apaH* Mutants Do Not Transcribe the *tar* Gene. To determine if AppppA was directly affecting σ^F RNA polymerase activity, crude extracts were prepared from a wild type and an *apaH*$^-$ mutant for *in vitro* transcriptions of pDNA1 template DNA. Primer extension analysis was used to detect *tar* transcripts generated *in vitro*. Fig. 5 shows that while extracts from *apaH*$^+$ produce abundant *tar* transcripts, *apaH*$^-$ cells do not. When AppppA was added back to wild-type extracts or to a purified σ^F transcription system, it had no inhibitory effect on transcription of the *tar* gene. Similarly, when extracts from wild-type and *apaH*$^-$ cells were mixed, there was no obvious inhibition of *tar* transcription by the wild-type extract. These results indicate that AppppA does not inhibit the activity of σ^F holoenzyme, but rather may affect its production in the *apaH*$^-$ background.

Effect of an *apaH* Mutation on Production of σ^F. To determine if *apaH*$^-$ mutants showed altered expression of *flbB* and *flaI*, which are thought to encode σ^F (D.N.A. and M.J.C., unpublished results), β-galactosidase levels were measured in *flaI*::*lacZ* fusion strains. Table 2 shows that transcription of *flaI* in *apaH*$^-$ cells is only 11% of wild type, and it is restored in *apaH*$^-$ cells carrying *apaH*$^+$ on an F′ episome. Furthermore, when *apaH*$^-$ mutants were transformed with a high-copy-number plasmid bearing *flbB/flaI* (pPM61), motility was restored (Fig. 3), possibly due to an increase in σ^F levels. Finally, 2-D protein gels were prepared with wild-type, *apaH*$^-$, and *flaI*$^-$ cells. *flaI*$^-$ mutants lack many of the same proteins missing or decreased in the *apaH*$^-$ mutant (data not shown). These results show that an *apaH*$^-$ mutation results in decreased motility and chemotaxis gene products by decreasing σ^F, and that these effects can be reversed by complementation with an *apaH*$^+$ allele or when *flbB/flaI* gene copy number is increased.

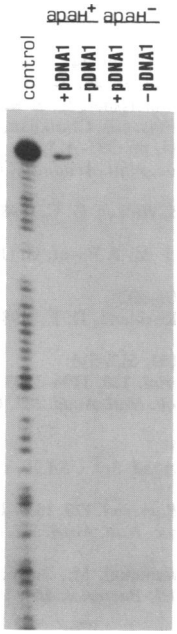

FIG. 4. Primer extension from *tar* gene transcripts from *apaH*$^+$ (AB1157) and *apaH*$^-$ (SF436) cells carrying pDNA1. Total RNA was isolated from wild-type and *apaH*$^-$ cells with and without pDNA1. RNA was mixed with a ^{32}P-labeled oligonucleotide, 5′-GCCAAAAACGGCCAAAACCAGCAG-3′, that is homologous to an interior sequence of the *tar* gene. The primers were extended as described (26), and the extension products were electrophoresed on a 6% polyacrylamide sequencing gel. Control is *in vitro* transcription of pDNA1 followed by primer extension with reverse transcriptase and labeled oligonucleotide. The arrow indicates fully extended labeled primer.

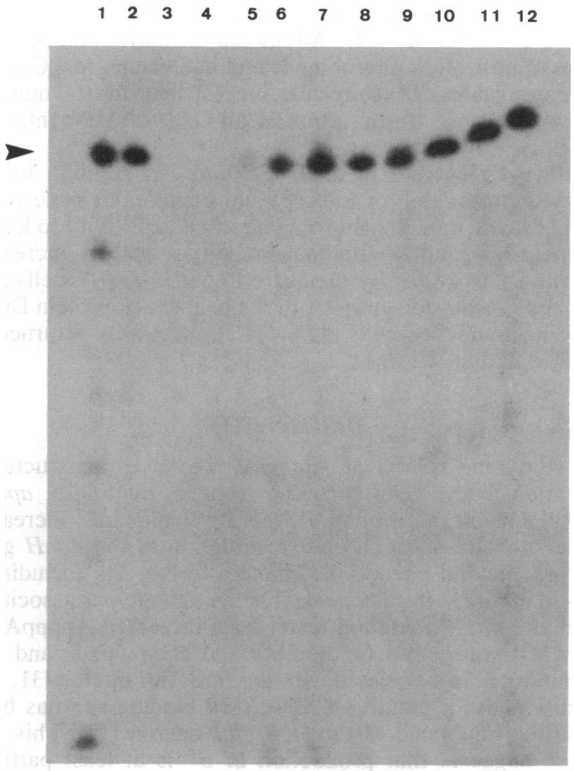

FIG. 5. *In vitro* transcription of *tar* gene on pDNA1 analyzed by primer extension. Crude extract or purified σ^F RNA polymerase with and without exogenous AppppA was added to transcription buffer, pDNA1, and 1 mM each dNTP, and the mixture was incubated 1 min at 30°C. The reaction was stopped by addition of phenol and chloroform/isoamyl alcohol, and then RNA was precipitated with ethanol and dried. A 5′ ^{32}P-labeled DNA primer, 5′-GCCAAAAACGGCCAAAACCAGCAG-3′, complementary to the *tar* gene mRNA synthesized *in vitro* was used to detect *tar* mRNA by primer extension (26). Reaction mixtures were extracted with phenol/chloroform prior to electrophoresis. Primer extension products were electrophoresed on a 6% polyacrylamide sequencing gel. The arrowhead indicates fully extended labeled primer. Lane 1, *apaH*$^+$ extract, 150 μg of protein; lane 2, *apaH*$^+$, 45 μg of protein; lane 3, *apaH*$^-$, 150 μg of protein; lane 4, *apaH*$^-$, 45 μg of protein; lane 5, 1:9 mixture of *apaH*$^+$ and *apaH*$^-$, total 150 μg of protein; lane 6, 1:1 mixture of *apaH*$^+$ and *apaH*$^-$, total 150 μg of protein; lane 7, 9:1 mixture of *apaH*$^+$ and *apaH*$^-$, total 150 μg of protein; lane 8, *apaH*$^+$ extract + 100 μM AppppA, 150 μg of protein; lane 9, *apaH*$^+$ extract + 1 mM AppppA, 150 μg of protein; lane 10, purified σ^F RNA polymerase; lane 11, purified σ^F RNA polymerase + 100 μM AppppA; lane 12, purified σ^F RNA polymerase + 1 mM AppppA.

The *apaH* Mutation Affects Catabolite Repression. Since there is a putative CAP–cAMP binding site upstream of the *flbB/flaI* operon (18), and since transcription of this operon is decreased in *apaH*$^-$ mutants, we assayed other catabolite-repressible gene activity. β-Galactosidase and galactokinase levels were measured in wild-type (SF43) and *apaH*$^-$ (SF502) cells grown in M9 salts plus 0.5% Casamino acids with 0.1 mM isopropyl β-D-thiogalactoside or 0.3% galactose added 45 min prior to assaying activity. *apaH*$^-$ mutants showed only 16% of wild-type β-galactosidase activity (764 vs. 4780

units) and only 16% of wild-type galactokinase activity (7219 vs. 45,113 cpm; $n = 3$). Additionally, *apaH*$^-$ showed decreased utilization of arabinose and maltose on MacConkey indicator plates. These results suggest that *apaH*$^-$ mutants show decreased transcription of all CAP–cAMP-controlled genes.

Other Phenotypes of *apaH* Mutants. In addition to decreased production of motility and chemotaxis gene products, *apaH*$^-$ mutants showed increased sensitivity to killing by near-UV and *N*-ethylmaleimide, as well as increased sensitivity to killing by exposure to 55°C. *apaH*$^-$ cells also showed prolonged synthesis of the heat shock protein DnaK after heat-shocked cells (43°C for 10 min) were returned to 30°C (data not shown).

DISCUSSION

To study the role(s) of AppppA, we have constructed a mutation in the gene encoding AppppA hydrolase, *apaH*, with the result that AppppA levels are significantly increased under nonstress conditions. A mutation in the *apaH* gene imparts several phenotypic changes to the cell, including a loss in motility. It is probable that the phenotypes associated with an *apaH*$^-$ mutation result from increased AppppA.

cAMP is required for motility and chemotaxis, and this requirement is associated with the *flbB/flaI* operon (31, 32). Additionally, a putative CRP–cAMP binding site has been identified upstream of the *flbB/flaI* operon (18). This evidence suggests that production of σ^F is at least partially controlled by CAP–cAMP. *apaH*$^-$ mutants show a similar decrease in transcription of *flbB/flaI*, *lacZ*, and *galK* (11%, 16%, and 16%, respectively). We think that these effects are a direct result of increased AppppA in *apaH*$^-$ mutants and that high AppppA levels cause decreased transcription of catabolite-repressible genes by altering the production or function of the CAP–cAMP complex. Addition of exogenous cAMP does not restore motility or induction of the *lacZ* gene in *apaH*$^-$ mutants, suggesting that it is CAP synthesis or function that is altered in the mutants.

Nucleotides other than cAMP can affect CAP function. CAP* mutants will utilize cGMP in place of cAMP (34), and ppGpp alters CAP function *in vitro* (35). Additionally, *crp* mutants produce β-galactosidase at only 15% the level of wild type when induced with isopropyl thiogalactoside. It is therefore possible that AppppA may interact with CAP to affect its function.

We have found that *apaH*$^-$ mutants do not constitutively induce the heat shock or OxyR response in spite of up to a 100-fold increase in the basal level of AppppA. Consistent with previous results (5, 12), this finding shows that AppppA cannot be the signal for the induction of the OxyR or heat shock responses.

This leaves open to question the function of AppppA production when wild-type cells are heat shocked or oxidatively stressed. It is possible that alteration of CAP–cAMP function is simply the means by which AppppA in heat-shocked or oxidatively stressed cells inhibits motility and that inhibition of transcription of other CAP–cAMP genes is a secondary effect. Alternatively, the effect of AppppA on motility may be a by-product of the more important function of down-regulating catabolite-repressible genes in general. Oxidative stress alters membrane integrity and leads to a transient loss of the protonmotive force (36). It is possible that AppppA is produced when the electron transport chain has been altered (i.e., O_2^- production by cytochrome oxidase increases). Transient inhibition of carbon source uptake and utilization would decrease oxidative phosphorylation and limit the further production of oxygen radicals.

Alternatively, CAP–cAMP has been shown to play regulatory roles in transcription termination (37) and cell division (38), and AppppA may function to alter these processes when the cell is exposed to stress. Finally, it is possible that AppppA and CAP form a complex required for up-transcription of genes important in responding to oxidative stress. This might account for the seven proteins showing elevated synthesis in *apaH*$^-$ mutants by 2-D gel analysis.

While we do not yet know the exact mechanism by which an *apaH*$^-$ mutation alters CAP–cAMP function, it has become clear from this work that it has a powerful effect upon cell metabolism and motility.

We thank S. Blanquet, P. Matsumura, Y. Komeda, and B. Bachman for plasmids and strains. S.B.F. was supported by National Institutes of Health Postdoctoral Fellowship ES05441-01. D.N.A. and M.J.C. were supported by a research grant (GM12010) from the National Institute of General Medical Sciences, and B.N.A. was supported by a National Cancer Institute Outstanding Investigator Grant (CA39910). This work was also supported by National Institute of Environmental Health Sciences Center Grant ES01896.

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