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Protein acetylation in prokaryotes increases stress resistance

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ABSTRACT

Acetylation of lysine residues is conserved in all three kingdoms; however, its role in prokaryotes is unknown. Here we demonstrate that acetylation enables the reference bacterium *Escherichia coli* to withstand environmental stress. Specifically, the bacterium reaches higher cell densities and becomes more resistant to heat and oxidative stress when its proteins are acetylated as shown by deletion of the gene encoding acetyltransferase YfiQ and the gene encoding deacetylase CobB as well as by overproducing YfiQ and CobB. Furthermore, we show that the increase in oxidative stress resistance with acetylation is due to the induction of catalase activity through enhanced *katG* expression. We also found that two-component system proteins CpxA, PhoP, UvrY, and BasR are associated with cell catalase activity and may be responsible as the connection between bacterial acetylation and the stress response. This is the first demonstration of a specific environmental role of acetylation in prokaryotes.

1. Introduction

The post-translational modification of acetylation occurs in all three domains of life [1] and regulates diverse aspects of metabolism in that 2700 proteins in mammals are acetylated related to central metabolism, mRNA splicing, protein synthesis, cell morphology, and cell cycle [2]. Although identified in 1963 for eukaryotes [2], in bacteria, the role of acetylation has not been well characterized even though this modification is relatively common in that at least 91 proteins are acetylated in the best-studied strain, *Escherichia coli,* including the stress related heat shock proteins like DnaK and superoxide dismutase [3,4].

In *Salmonella enterica*, there is only one major bacterial protein acetyltransferase, Pat, and one nicotinamide adenine dinucleotide-dependent deacetylase, CobB. These two enzymes control the status of lysine acetylation for acetyl-CoA synthetase as well as the acetylation of a number of central metabolic enzymes in *S. enterica* [5]. In *E. coli*, there are 23 putative lysine acetyltransferases that add acetyl groups to the epsilon amine of lysine using acetyl-coenzyme A as a substrate [1]. Ten Gcn-5 acetyltransferases in *E. coli* are confirmed for their function while the other thirteen remain enigmatic, including YfiQ which is the homolog of the single acetyltransferase in *S. enteric* [1]. Hence we chose to study YfiQ as the acetyltransferase because we expect that it also plays an important role in *E. coli* similar to Pat in *S. enteric* [1] and chose to study CobB as the deacetylase since it is the only confirmed deacetylase in *E. coli* [6]. Our goal was to determine the role of YfiQ and CobB in bacterial physiology by changing acetylation in *E. coli*.

Bacteria respond to various stresses by producing global regulators [7]. The universal stress proteins UspA and UspD are required for resistance to superoxide-generating agents [8]. Similarly, OxyR regulates the peroxide-mediated stress response in which at least 30 proteins are elevated over the basal levels upon the addition of peroxide stress [7]. In addition, the sigma factor RpoS regulates *katG* which encodes catalase in an OxyR-dependent way [9]. RpoS is also required for acid, heat, and salt resistance in *E. coli* O157:H7 [10].

Since the ability of bacteria to respond rapidly to stress is a hallmark of their success and since protein modifications allow the most rapid response, we hypothesized that conserved protein acetylation may be related to the ability of the cell to withstand stress. Here we demonstrate that cells with decreased acetylation, through enhanced deacetylase CobB activity, are less resistant to heat and oxidative stress. Both a whole-transcriptome analysis and quantitative, reverse-transcription polymerase chain reaction (qRT-PCR) showed repression of *katG* under oxidative stress conditions when CobB is produced. Furthermore, we found that the two-component regulator proteins CpxA, UvrY, PhoP, and BasR are related to catalase activity and may work as the acetylation targets, which can thus control the stress response of the cell. Hence, we propose that the activity of some regulators that control stress gene expression, especially *katG* expression, is altered by acetylation.

2. Materials and methods

2.1. Bacterial strains, plasmids, and growth conditions

The bacterial strains and plasmids used in this study are listed in Table 1. *E. coli* K-12 BW25113 and its isogenic mutants [11] were

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Table 1
E. coli strains and plasmids used in this study. Km^r and Cm^r denote kanamycin and chloramphenicol resistance, respectively.

Strain/plasmid	Genotype	Source
Strain		
BW25113	*lacI*^{q} *rrnB*$_{T14}$ Δ*lacZ*$_{WJ16}$ *hsdR514* Δ*araBAD*$_{AH33}$ Δ*rhaBAD*$_{LD78}$	[41]
BW25113 *cobB*	BW25113 Δ*cobB* Ω Km^r	[11]
BW25113 *yfiQ*	BW25113 Δ*yfiQ* Ω Km^r	[11]
BW25113 *katE*	BW25113 Δ*katE* Ω Km^r	[11]
BW25113 *cpxA*	BW25113 Δ*cpxA* Ω Km^r	[11]
BW25113 *cpxR*	BW25113 Δ*cpxR* Ω Km^r	[11]
BW25113 *barA*	BW25113 Δ*barA* Ω Km^r	[11]
BW25113 *uvrY*	BW25113 Δ*uvrY* Ω Km^r	[11]
BW25113 *basS*	BW25113 Δ*basS* Ω Km^r	[11]
BW25113 *basR*	BW25113 Δ*basR* Ω Km^r	[11]
BW25113 *phoQ*	BW25113 Δ*phoQ* Ω Km^r	[11]
BW25113 *phoP*	BW25113 Δ*phoP* Ω Km^r	[11]
BW25113 *rcsC*	BW25113 Δ*rcsC* Ω Km^r	[11]
BW25113 *rcsB*	BW25113 Δ*rcsB* Ω Km^r	[11]
BW25113 *arcB*	BW25113 Δ*arcB* Ω Km^r	[11]
BW25113 *arcA*	BW25113 Δ*arcA* Ω Km^r	[11]
BW25113 *evgS*	BW25113 Δ*evgS* Ω Km^r	[11]
BW25113 *evgA*	BW25113 Δ*evgA* Ω Km^r	[11]
Plasmid		
pCA24N	Cm^r; *lacI*^{q}, pCA24N	[12]
pCA24N_*cobB*	Cm^r; *lacI*^{q}, pCA24N $p_{T5\text{-}lac}$::*cobB*	[12]
pCA24N_*yfiQ*	Cm^r; *lacI*^{q}, pCA24N $p_{T5\text{-}lac}$::*yfiQ*	[12]
pCA24N_*rpoS*	Cm^r; *lacI*^{q}, pCA24N $p_{T5\text{-}lac}$::*rpoS*	[12]
pCA24N_*katG*	Cm^r; *lacI*^{q}, pCA24N $p_{T5\text{-}lac}$::*katG*	[12]
pCA24N_*katE*	Cm^r; *lacI*^{q}, pCA24N $p_{T5\text{-}lac}$::*katE*	[12]

obtained from the Genome Analysis Project in Japan. Plasmids pCA24N_*cobB* and pCA24N_*yfiQ*, carrying *cobB* and *yfiQ* under control of the $P_{T5\text{-}lac}$ promoter with tight regulation via the *lacI*^{q} repressor, and the empty plasmid pCA24N were also obtained from the Genomic Analysis Project in Japan [12]. Expression of *cobB* and *yfiQ* was induced by 0.1 mM isopropyl-β-*D*-thiogalactopyranoside (IPTG) (Sigma, St. Louis, MO). All experiments were conducted in Luria–Bertani medium (LB) medium [13] at 37 °C unless indicated otherwise. Kanamycin (50 μg/mL) was used for pre-culturing the isogenic knock-outs. Chloramphenicol (30 μg/mL) was used for maintaining the pCA24N-based plasmids.

For the growth tests, overnight cultures were diluted to a turbidity of 0.05 at 600 nm and grown for 2 h, then 0.1 mM IPTG was used to induce *cobB* and *yfiQ* expression. One millilitre of culture was taken out at each time point, and the cell turbidity at 600 nm was measured.

2.2. Stress assays

Overnight cultures were diluted to a turbidity of 0.05 at 600 nm and grown for 2 h. Then 0.1 mM IPTG was used to induce *cobB* and *yfiQ* expression for 10 to 12 h. Cells were centrifuged and resuspended in phosphate buffered saline (PBS) to a turbidity of 1.0. For the heat resistance assay, samples were treated at 65 °C for 20 min [14]. For the H_2O_2 resistance assay, samples were mixed with 20 mM H_2O_2 for 20 min [15].

2.3. Catalase assays

Overnight cultures were diluted to a turbidity of 0.05 at 600 nm and grown for 2 h. IPTG (0.1 mM) was added, and the cultures were grown for another 4 h. Catalase activity was then measured two different ways in this study. For the spectrophotometric assay [16], 1 mL of culture for each sample was washed and resuspended into PBS buffer for a turbidity at 600 nm of 0.5. For each sample, catalase activity was measured by checking the rate of H_2O_2 decrease which is reflected by the rate of change of absorbance at 240 nm at 37 °C. Two tubes were used for each sample, and 5 mM H_2O_2 was added for one while the other one was used as a control. The conversion between H_2O_2 concentration and absorbance was that 10 mM H_2O_2 is equal to an optical density (OD) of 0.36 at 240 nm [16]. For the catalase activity of the two-component system mutants, overnight cultures for each strain were diluted to a turbidity of 0.05 at 600 nm and grown until a turbidity at 600 nm of 1.0. One millilitre of culture for each sample was taken and catalase activity was measured as described above.

The second method for catalase activity used a colorimetric assay with dicarboxidine/lactoperoxidase [17]. The cultures were washed and resuspended in M9 glucose medium for a turbidity of 1.0 at 600 nm. IPTG (0.1 mM) was added to each sample to induce the expression of *cobB* and *yfiQ*. The cultures were then incubated for 4 h. Before the test, a solution of 50 μg/mL of lactoperoxidase was mixed with an equal volume of 1 mM dicarboxidine solution in water (dicarboxidine is converted into a yellow product in a reaction catalyzed by the activity of lactoperoxidase, and the amount of color developed is directly proportional to the amount of H_2O_2 present in the medium). To start the reaction, 10 mM H_2O_2 was added to the culture, and samples (10 μL) were taken after every 2 min at the beginning and 5–10 min later. The samples were added to a 200 μL reaction mixture, and the absorbance (OD 450 nm) was measured immediately. Higher catalase activity causes a faster decrease in the OD 450 nm.

2.4. qRT-PCR

qRT-PCR was performed using the StepOne™ Real-Time PCR System (Applied Biosystems, Foster City, CA). After isolating RNA [18] using RNA*later*™ (Ambion, Austin, TX), 50 ng of total RNA was used for the qRT-PCR reaction using the *Power* SYBR® Green RNA-to-C_T™ *1-Step* Kit (Applied Biosystems). The primers are listed in Table 2. The housekeeping gene *rrsG* was used to normalize the gene expression data [19]. The annealing temperature was 60 °C for all the genes in this study. To investigate the transcription level of *rpoS*, *katG*, and *katE* under oxidative stress conditions, overnight cultures were diluted to a turbidity of 0.05 at 600 nm, grown for 2 h, 0.1 mM IPTG was added for another 4 h to induce *cobB* and *yfiQ* expression, and the cells were exposed to 20 mM H_2O_2 for 10 min.

2.5. Whole-transcriptome analysis

Overnight cultures of *cobB*/pCA24N_*cobB* and *cobB*/pCA24N were diluted to a turbidity of 0.05 at 600 nm, grown for 2 h, 0.1 mM IPTG was added for another 4 h to induce *cobB* expression, and the cells were exposed to 20 mM H_2O_2 for 10 min. Cell pellets were collected and resuspended in RNA*later* (Ambion Inc., Austin, TX), and total RNA was isolated using the RNeasy Mini Kit (Qiagen Inc., Valencia, CA) [18]. The *E. coli* GeneChip Genome

Table 2
Primers used for qRT-PCR in this study.

Primer name	Sequence
rpoS-f	5′-AGAGTAACTTGCGTCTGGTGGTAAA-3′
rpoS-r	5′-ATAGTACGGGTTTGGTTCATAATCG-3′
katG-f	5′-CTGGTGTGGTTGGTGTTGAG-3′
katG-r	5′-AGTGACTCGGTGGTGGAAAC-3′
katE-f	5′-GATCTTCTCGATCCAACCAAAC-3′
katE-r	5′-CACCAAGACGACTGATTTGTGT-3′
rrsG-f	5′-TATTGCACAATGGGCGCAAG-3′
rrsG-r	5′-ACTTAACAAACCGCTGCGT-3′

2.0 array (Affymetrix, P/N 900551) was used, and cDNA synthesis, fragmentation, and hybridizations were performed as described previously [20]. If the gene with the larger transcription rate did not have a consistent transcription rate based on the 11–15 probe pairs (*P*-value less than 0.05), these genes were discarded. A gene was considered differentially expressed when the *P*-value for comparing two chips was lower than 0.05 (to assure that the change in gene expression was statistically significant and that false positives arise less than 5%) and if their fold change is higher than standard deviation for the whole genome [21]. The data have been deposited at the NCBI Gene Expression Omnibus (GEO ID: GSE29803).

2.6. Mass spectrometry (MS)

His-tagged RpoS, KatG, and KatE were purified from pCA24N-based plasmids in the *cobB* mutant (to purify potential acetylated proteins) and in the *yfiQ* mutant (to purify the potential unacetylated proteins). Overnight cultures were diluted in 1 L to a turbidity of 0.05 at 600 nm. Each sample was grown until the cell turbidity at 600 nm reached 0.6–1.0. Cultures were kept at 4 °C for 45 min and then induced with 0.1 mM IPTG overnight at room temperature. Cell pellets were collected and lysed with a French Press. Supernatants were treated with 1 mL Ni–NTA agarose resin for 2 h. Purified His-tag proteins were digested with trypsin and cleaned with a zip-tip cleaning method. Samples were eluted with 4 μL 0.1% formic acid plus 25% and 50% acetonitrile. The MS results were obtained using 4800 MALDI TOF/TOF Analyzer (Applied Biosystems/MDS Sciex, Carlsbad, CA) [22].

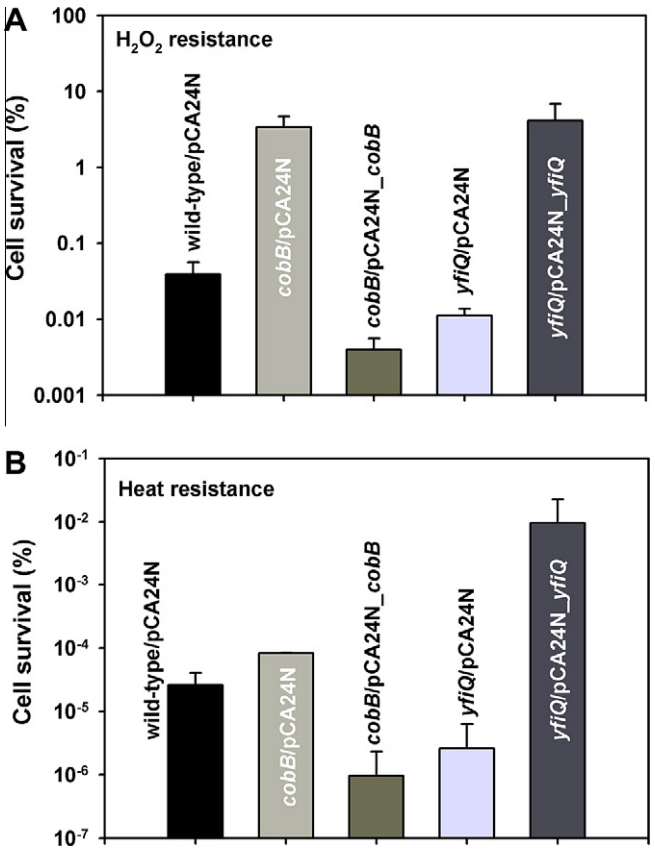

Fig. 1. Resistance to oxidative and heat stress. *E. coli* BW25113 wild-type/pCA24N, *cobB*/pCA24N, *cobB*/pCA24N_*cobB*, *yfiQ*/pCA24N, and *yfiQ*/pCA24N_*yfiQ* were tested for H_2O_2 resistance (A) and heat resistance (B) in LB medium at 37C.

3. Results

3.1. YfiQ increases stress resistance and CobB decreases stress resistance

To test our hypothesis that lysine acetylation is related to stress resistance, we subjected *E. coli yfiQ* and *cobB* mutants to oxidative and heat stress (Fig. 1); the rationale was that inactivation of acetyltransferase YfiQ should decrease acetylation and inactivation of deacetylase CobB should increase acetylation of all the cell proteins. We found that decreasing acetylation by deleting *yfiQ* decreased oxidative and heat stress by 4-fold and 10-fold, respectively, while increasing acetylation by deleting *cobB* increased oxidative and heat stress resistance by 100 fold and 3-fold, respectively. These results were complemented by increasing acetylation by producing YfiQ via plasmid pCA24N_*yfiQ* (110-fold increase in oxidative stress resistance and 370-fold increase in heat resistance) and by decreasing acetylation by producing CobB via plasmid pCA24N_*cobB* (10-fold reduction in oxidative stress resistance and 27-fold reduction in heat resistance). Therefore, acetylation by YfiQ increases resistance to heat and oxidative stress while deacetylation by CobB decreases it. Hence, protein acetylation helps the cell respond to environmental stress.

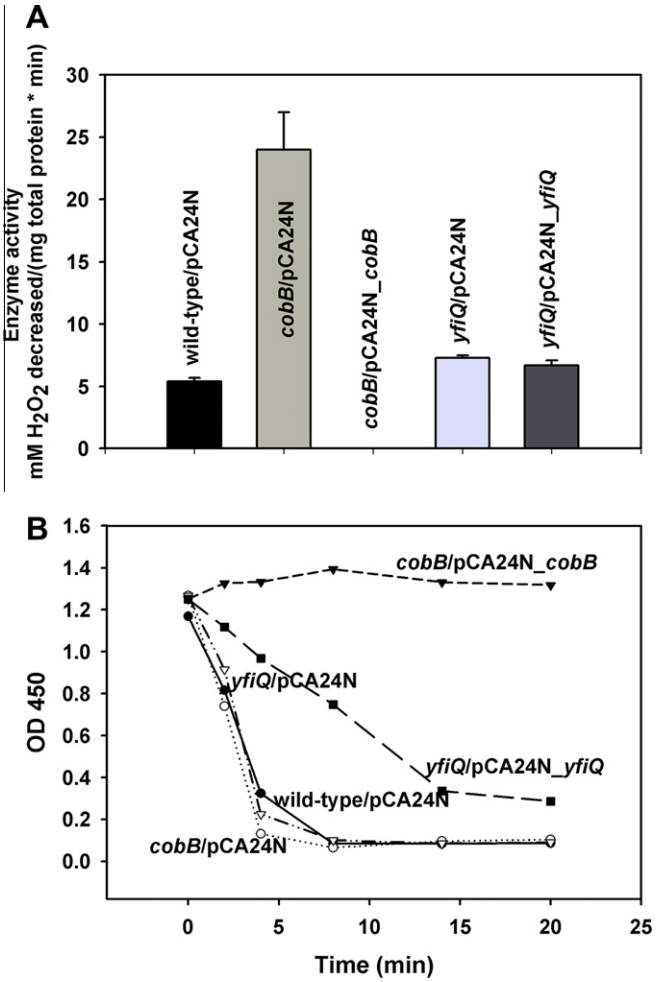

Fig. 2. Catalase activity. *E. coli* BW25113 wild-type/pCA24N, *cobB*/pCA24N, *cobB*/pCA24N_*cobB*, *yfiQ*/pCA24N, and *yfiQ*/pCA24N_*yfiQ* were compared for their catalase activity using the a spectrophotometric method (A) and a colorimetric method with dicarboxidine/lactoperoxidase (B). Wild-type/pCA24N data are indicated by filled circles, *cobB*/pCA24N by empty circles, *cobB*/pCA24N_*cobB* by filled triangles, *yfiQ*/pCA24N by empty triangles, and *yfiQ*/pCA24N_*yfiQ* by solid squares.

3.2. CobB decreases catalase activity

Since producing CobB decreased resistance to hydrogen peroxide (Fig. 1A), we investigated whether this phenotype was related to RpoS since RpoS is a positive regulator of catalase activity via *katG* and *katE* [23]. Catalase deactivates H_2O_2 by converting it to H_2O and O_2 [24]. Using two independent assays, we found that removing acetylation by producing CobB abolished catalase activity and increasing acetylation by deleting *cobB* increased catalase activity by 4.4-fold (Fig. 2). Therefore, acetylation increases catalase activity. However, the catalase activity was not changed much by producing YfiQ (Fig. 2). This is probably because of the contribution of the other nine functional acetyltransferases in *E. coli* [1].

3.3. Catalase related proteins KatG, KatE, and RpoS are not acetylated

Since catalase activity was affected by acetylation (Fig. 2), we investigated whether catalases KatG and KatE, as well as the regulator that controls catalase gene expression, RpoS, are acetylated. After analyzing around 100 peptide sequences, the MS results indicated that none of these proteins are directly acetylated on lysine residues. Hence, the KatG, KatE, and RpoS are all not acetylated, and the increased catalase activity by acetylation is not because of direct modification of these three proteins.

3.4. Catalase genes are induced by acetylation

To check if there are any differences in transcription of the catalase genes caused by acetylation, we measured gene expression via qRT-PCR for *rpoS*, *katG*, and *katE* (Table 3). *katG* was repressed 3.8 ± 0.3-fold in the *cobB*/pCA24N_*cobB* strain compared to the *cobB*/pCA24N strain without H_2O_2 addition. With the addition of 20 mM H_2O_2 for 10 min, *katG* was repressed even more (25 ± 2-fold) for *cobB*/pCA24N_*cobB* vs. *cobB*/pCA24N. Hence, catalase genes are induced due to acetylation of some unknown cellular proteins. Probably stress activates some regulator via a post-translational modification which leads to induction of *katG*.

3.5. Acetylation induces the transcription of genes involved for various stresses

To analyze the global effect of acetylation on gene transcription, a whole-transcriptome analysis was performed with the *cobB*/pCA24N_*cobB* strain vs. the *cobB*/pCA24N strain with the rationale that production of CobB should remove the acetyl groups on all the cell proteins. We found that in addition to *katG* and *katE,* various stress-related genes are repressed by deacetylation, including the heat shock genes *dnaK* [25], osmotic stress genes *osmB* [26] and *osmY* [27], acid resistance genes *gadABCE* and *hdeABD* [28], cold shock genes *cspAB* [29], carbon starvation gene *csiD* and *slp* [30], and general stress gene *yhbO* [31] (Table 4). Hence, protein acetylation is involved in various bacterial stress response systems.

3.6. Two-component systems may work as the targets for acetylation

Since various stress genes changed expression upon acetylation (Table 4), we tested 14 mutants from all major two-component systems in *E. coli* for their catalase activity to see if any of these two-component system proteins can directly change catalase activity and can work as the targets for acetylation. The two-component systems tested included CpxA/CpxR (heat shock regulon [32]), BarA/UvrY (related to oxidative stress [33]), BasS/BasR (related to iron response [34]), PhoQ/PhoP (related to acid resistance response [35]), RcsC/RcsB (regulate cell division genes [36]), ArcB/ArcA (related to resistance to reactive oxygen stress [37]), and EvgS/EvgA (related to acid resistance and multidrug resistance [38]). Of these, we found that the *cpxA*, *uvrY*, *phoP*, and *basR* mutants showed less catalase activity than the other mutants (Fig. 3). Hence we hypothesize that proteins CpxA, UvrY, PhoP, and BasR may work as the targets for acetylation by YfiQ and control bacterial stress response depending on their acetylation state.

3.7. YfiQ increases growth yield and CobB decreases it

We also found that cells with acetylation from producing YfiQ had a dramatically increased growth yield in rich medium while cells that lacked acetylation had reduced yield (Fig. 4). Therefore, acetylation helps cells cope with the stress associated with stationary phase growth.

4. Discussion

Previously, the stress proteins heat shock protein DnaK [4], heat shock chaperone HtpG, superoxide dismutase SodA [4], SodB [3], alkylhydroperoxide reductase AhpC [3], and thioredoxin TrxA [3] were found to be acetylated in *E. coli*. However, there has been no prior report connecting the post-translational modification of acetylation and resistance to any environmental stress, and there has been no connection made between acetylation and a specific stress pathway. We discovered here that acetylation plays a significant role in the resistance to both oxidative stress and heat resistance; therefore, we connected protein acetylation and these two environmental stresses. We also found through a whole-transcriptome approach that acetylation controls an even broader range of stresses by altering expression of genes related to stress resistance (including osmotic, acid, cold, and carbon starvation); this is the first whole-transcriptome study for acetylation. Hence, here we connected protein acetylation to the resistance of specific stresses

Table 3
qRT-PCR results for catalase-related genes *rpoS*, *katG*, and *katE*. ΔCt is the threshold difference between each gene and the housekeeping gene *rrsG*. Fold change indicates the gene transcription difference between the *cobB*/pCA24N_*cobB* strain vs. the *cobB*/pCA24N strain in LB medium at 37 °C with 20 mM H_2O_2 for 10 min. Fold changes are relative to the *cobB*/pCA24N sample.

Strain name	***rpoS***		***katG***		***katE***	
	ΔCt	Fold change	ΔCt	Fold change	ΔCt	Fold change
No stress						
cobB/pCA24N	12.36 ± 0.07	1.00	8.96 ± 0.07	1.00	11.8 ± 0.1	1.00
cobB/pCA24N_*cobB*	11.8 ± 0.1	1.47	10.90 ± 0.07	−3.84	12.48 ± 0.04	−1.60
1 min H_2O_2 treatment						
cobB/pCA24N	11.0 ± 0.3	1.00	7.7 ± 0.3	1.00	10.1 ± 0.3	1.00
cobB/pCA24N_*cobB*	13.5 ± 0.1	−5.66	11.87 ± 0.07	−18.00	13.03 ± 0.08	−7.62
10 min H_2O_2 treatment						
cobB/pCA24N	11.7 ± 0.1	1.00	7.4 ± 0.3	1.00	10.9 ± 0.3	1.00
cobB/pCA24N_*cobB*	13.2 ± 0.2	−2.83	12.05 ± 0.09	−25.11	13.1 ± 0.1	−4.59

Table 4
Summary of the whole-transcriptome results showing the stress genes that are repressed by production of CobB. Fold change indicates the gene transcription difference between the *cobB*/pCA24N_*cobB* strain vs. the *cobB*/pCA24N strain when cultured in LB medium at 37 °C with 20 mM H_2O_2 for 10 min and with 0.1 mM IPTG to induce production of CobB.

Gene	Fold change	Gene function
dnaK	−4.0	Chaperone Hsp70; DNA biosynthesis; autoregulated heat shock proteins
osmB	−3.3	Osmotically inducible lipoprotein
osmY	−3.0	Hyperosmotically inducible periplasmic protein
gadC	−12.1	Predicted glutamate-GABA antiporter; glutamate-dependent enzyme, may function in protection against cytoplasmic acidification
gadB	−18.4	Glutamate decarboxylase isozyme
gadE	−6.5	Transcriptional regulator of the gadABC operon
gadA	−14.9	Glutamate decarboxylase A; RpoS regulon. EvgAS regulon. H-NS repressed. Induced by acid shock and salt stress.
cspB	−3.2	Cold shock protein
cspA	−3.0	Cold shock protein
katE	−3.7	Catalase; hydroperoxidase HPII(III)
katG	−4.9	Catalase; hydroperoxidase HPI(I)
csiD	−3.2	Carbon starvation induced gene
yhbO	−3.5	Stress-resistance protein, protease homolog
slp	−11.3	Outer membrane protein induced after carbon starvation
hdeB	−16.0	Periplasmic chaperone of acid-denatured proteins; H-NS repressed
hdeA	−14.9	Periplasmic chaperone of acid-denatured proteins; H-NS repressed
hdeD	−7.5	Putative membrane transporter, H-NS repressed

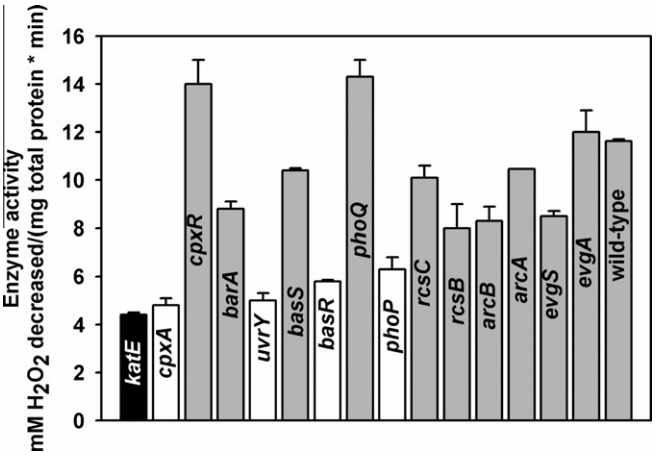

Fig. 3. Catalase activity for mutants of two-component systems. Single mutants from seven sets of two-component systems were compared for their catalase activity using the spectrophotometric method. The *katE* mutant was used as a negative control.

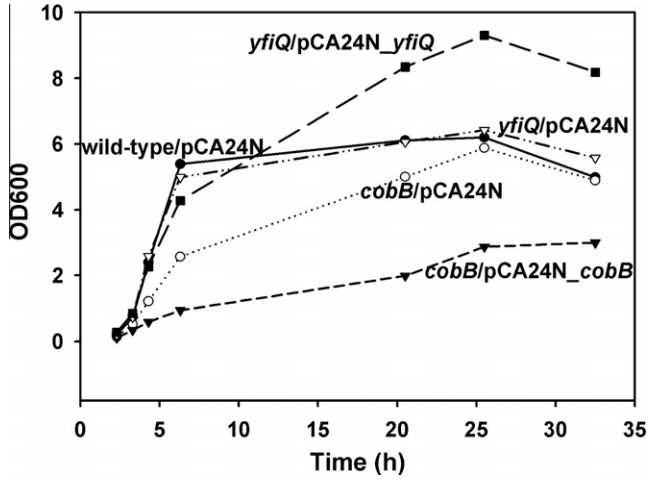

Fig. 4. Growth curves. The strains were grown in LB medium with 30 μg/mL chloramphenicol to retain the plasmids at 37 °C, and 0.1 mM IPTG was added to induce *cobB* and *yfiQ* expression after 2 h. Symbols as defined in Fig. 2B.

(i.e., showed acetylation is involved in both the response to oxidative and heat stress as well as linked acetylation to osmotic, acid, cold, and carbon starvation) and showed that for oxidative stress, the resistance stems from changes in transcription of the *katG* gene which encodes catalase.

Bacteria sense some stresses via two-component systems and then regulate the expression of various genes. The survey of seven sets of two-component system proteins showed that CpxA, UvrY, PhoP, and BasR are involved in the cell stress response to oxidative stress (Fig. 3); therefore, these proteins are likely to be involved in the acetylation response required for catalase activity. Since stress resistance genes are activated less in the CobB overproduction strain (due to deacetylation), we predict that one or more of these two-component proteins (a sensor or regulator) or the essential genes that the regulator directly controls, needs acetylation to respond to oxidative stress. Previously the two-component system protein RcsB was found to be acetylated in *E. coli* [39]. However, in our results, we did not see a large effect of RcsB on catalase activity (Fig. 3). Since the RcsB/RcsC two-component system is required for acid resistance [40], it is also possible that RcsB may work with other two-component system proteins after acetylation and thus change the cell physiology.

Acknowledgments

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References

[1] J.C. Escalante-Semerena, Nϵ–lysine acetylation control conserved in all three life domains, Microbe 5 (2010) 340–344.
[2] L.I. Hu, B.P. Lima, A.J. Wolfe, Bacterial protein acetylation: the dawning of a new age, Mol. Microbiol. 77 (2010) 15–21.
[3] J. Zhang, R. Sprung, J. Pei, X. Tan, S. Kim, H. Zhu, C.F. Liu, N.V. Grishin, Y. Zhao, Lysine acetylation is a highly abundant and evolutionarily conserved modification in *Escherichia coli*, Mol. Cell Proteomics 8 (2009) 215–225.
[4] B.J. Yu, J.A. Kim, J.H. Moon, S.E. Ryu, J.G. Pan, The diversity of lysine-acetylated proteins in *Escherichia coli*, J. Microbiol. Biotechnol. 18 (2008) 1529–1536.
[5] Q. Wang, Y. Zhang, C. Yang, H. Xiong, Y. Lin, J. Yao, H. Li, L. Xie, W. Zhao, Y. Yao, Z.B. Ning, R. Zeng, Y. Xiong, K.L. Guan, S. Zhao, G.P. Zhao, Acetylation of metabolic enzymes coordinates carbon source utilization and metabolic flux, Science 327 (2010) 1004–1007.
[6] R. Li, J. Gu, Y.Y. Chen, C.L. Xiao, L.W. Wang, Z.P. Zhang, L.J. Bi, H.P. Wei, X.D. Wang, J.Y. Deng, X.E. Zhang, CobB regulates *Escherichia coli* chemotaxis by deacetylating the response regulator CheY, Mol. Microbiol. 76 (2010) 1162–1174.
[7] S.B. Farr, T. Kogoma, Oxidative stress responses in *Escherichia coli* and *Salmonella typhimurium*, Microbiol. Rev. 55 (1991) 561–585.

[8] L. Nachin, U. Nannmark, T. NystrÖm, Differential roles of the universal stress proteins of *Escherichia coli* in oxidative stress resistance, adhesion, and motility, J. Bacteriol. 187 (2005) 6265–6272.
[9] A. Ivanova, C. Miller, G. Glinsky, A. Eisenstark, Role of *rpoS* (*katF*) in *oxyR*-independent regulation of hydroperoxidase I in *Escherichia coli*, Mol. Microbiol. 12 (1994) 571–578.
[10] A.M. Cheville, K.W. Arnold, C. Buchrieser, C.M. Cheng, C.W. Kaspar, *rpoS* regulation of acid, heat, and salt tolerance in *Escherichia coli* O157:H7, Appl. Environ. Microbiol. 62 (1996) 1822–1824.
[11] T. Baba, T. Ara, M. Hasegawa, Y. Takai, Y. Okumura, M. Baba, K.A. Datsenko, M. Tomita, B.L. Wanner, H. Mori, Construction of *Escherichia coli* K-12 in-frame, single-gene knockout mutants: the Keio collection, Mol. Syst. Biol. 2 (2006) 2006–2008.
[12] M. Kitagawa, T. Ara, M. Arifuzzaman, T. Ioka-Nakamichi, E. Inamoto, H. Toyonaga, H. Mori, Complete set of ORF clones of *Escherichia coli* ASKA library (a complete set of *E. coli* K-12 ORF archive): unique resources for biological research, DNA Res. 12 (2005) 291–299.
[13] J. Sambrook, E.F. Fritsch, T. Maniatis, Molecular Cloning: a Laboratory Manual, 2nd ed., Cold Spring Harbor Laboratory Press, NY, 1989.
[14] X.S. Zhang, R. García-Contreras, T.K. Wood, YcfR (BhsA) influences *Escherichia coli* biofilm formation through stress response and surface hydrophobicity, J. Bacteriol. 189 (2007) 3051–3062.
[15] X. Wang, Y. Kim, S.H. Hong, Q. Ma, B.L. Brown, M. Pu, A.M. Tarone, M.J. Benedik, W. Peti, R. Page, T.K. Wood, Antitoxin MqsA helps mediate the bacterial general stress response, Nat. Chem. Biol. 7 (2011) 359–366.
[16] I. Gusarov, E. Nudler, NO-mediated cytoprotection: instant adaptation to oxidative stress in bacteria, Proc. Natl. Acad. Sci. USA 102 (2005) 13855–13860.
[17] M. Macvanin, D. Hughes, Assays of sensitivity of antibiotic-resistant bacteria to hydrogen peroxide and measurement of catalase activity, Methods Mol. Biol. 642 (2010) 95–103.
[18] D. Ren, L.A. Bedzyk, S.M. Thomas, R.W. Ye, T.K. Wood, Gene expression in *Escherichia coli* biofilms, Appl. Microbiol. Biotechnol. 64 (2004) 515–524.
[19] J. Lee, T. Maeda, S.H. Hong, T.K. Wood, Reconfiguring the quorum-sensing regulator SdiA of *Escherichia coli* to control biofilm formation via indole and *N*-acylhomoserine lactones, Appl. Environ. Microbiol. 75 (2009) 1703–1716.
[20] A.F. González Barrios, R. Zuo, Y. Hashimoto, L. Yang, W.E. Bentley, T.K. Wood, Autoinducer 2 controls biofilm formation in *Escherichia coli* through a novel motility quorum-sensing regulator (MqsR, B3022), J. Bacteriol. 188 (2006) 305–316.
[21] D. Ren, L.A. Bedzyk, R.W. Ye, S.M. Thomas, T.K. Wood, Differential gene expression shows natural brominated furanones interfere with the autoinducer-2 bacterial signaling system of *Escherichia coli*, Biotechnol. Bioeng. 88 (2004) 630–642.
[22] S.D. Sherrod, A.J. Diaz, W.K. Russell, P.S. Cremer, D.H. Russell, Silver nanoparticles as selective ionization probes for analysis of olefins by mass spectrometry, Anal. Chem. 80 (2008) 6796–6799.
[23] S. Lacour, P. Landini, SigmaS-dependent gene expression at the onset of stationary phase in *Escherichia coli*: function of SigmaS-dependent genes and identification of their promoter sequences, J. Bacteriol. 186 (2004) 7186–7195.
[24] V. Robbe-Saule, C. Coynault, M. Ibanez-Ruiz, D. Hermant, F. Norel, Identification of a non-haem catalase in *Salmonella* and its regulation by RpoS (σ^S), Mol. Microbiol. 39 (2001) 1533–1545.
[25] K. Seyer, M. Lessard, G. Piette, M. Lacroix, L. Saucier, *Escherichia coli* heat shock protein DnaK: production and consequences in terms of monitoring cooking, Appl. Environ. Microbiol. 69 (2003) 3231–3237.
[26] J.U. Jung, C. Gutierrez, F. Martin, M. Ardourel, M. Villarejo, Transcription of *osmB*, a gene encoding an *Escherichia coli* lipoprotein, is regulated by dual signals. Osmotic stress and stationary phase, J. Biol. Chem. 265 (1990) 10574–10581.
[27] R. Hengge-Aronis, R. Lange, N. Henneberg, D. Fischer, Osmotic regulation of *rpoS*-dependent genes in *Escherichia coli*, J. Bacteriol. 175 (1993) 259–265.
[28] J. Lee, R. Page, R. Garcia-Contreras, J.M. Palermino, X.S. Zhang, O. Doshi, T.K. Wood, W. Peti, Structure and function of the *Escherichia coli* protein YmgB: a protein critical for biofilm formation and acid-resistance, J. Mol. Biol. 373 (2007) 11–26.
[29] N.N. Ulusu, E.F. Tezcan, Cold shock proteins, Turk. J. Med. Sci. 31 (2001) 283–290.
[30] D.M. Alexander, A.C. St John, Characterization of the carbon starvation-inducible and stationary phase-inducible gene *slp* encoding an outer membrane lipoprotein in *Escherichia coli*, Mol. Microbiol. 11 (1994) 1059–1071.
[31] J. Abdallah, R. Kern, A. Malki, V. Eckey, G. Richarme, Cloning, expression, and purification of the general stress protein YhbO from *Escherichia coli*, Protein Expr. Purif. 47 (2006) 455–460.
[32] C. Cuny, M. Lesbats, S. Dukan, Induction of a global stress response during the first step of *Escherichia coli* plate growth, Appl. Environ. Microbiol. 73 (2007) 885–889.
[33] C.D. Herren, A. Mitra, S.K. Palaniyandi, A. Coleman, S. Elankumaran, S. Mukhopadhyay, The BarA-UvrY two-component system regulates virulence in avian pathogenic *Escherichia coli* O78:K80:H9, Infect. Immun. 74 (2006) 4900–4909.
[34] D. Hagiwara, T. Yamashino, T. Mizuno, A genome-wide view of the *Escherichia coli* BasS-BasR two-component system implicated in iron-responses, Biosci. Biotechnol. Biochem. 68 (2004) 1758–1767.
[35] B.L. Bearson, L. Wilson, J.W. Foster, A low pH-inducible, PhoPQ-dependent acid tolerance response protects *Salmonella typhimurium* against inorganic acid stress, J. Bacteriol. 180 (1998) 2409–2417.
[36] F. Carballes, C. Bertrand, J.P. Bouche, K. Cam, Regulation of *Escherichia coli* cell division genes *ftsA* and *ftsZ* by the two-component system *rcsC–rcsB*, Mol. Microbiol. 34 (1999) 442–450.
[37] C. Loui, A.C. Chang, S. Lu, Role of the ArcAB two-component system in the resistance of *Escherichia coli* to reactive oxygen stress, BMC Microbiol. 9 (2009) 183.
[38] Y. Eguchi, J. Itou, M. Yamane, R. Demizu, F. Yamato, A. Okada, H. Mori, A. Kato, R. Utsumi, B1500, a small membrane protein, connects the two-component systems EvgS/EvgA and PhoQ/PhoP in *Escherichia coli*, Proc. Natl. Acad. Sci. USA 104 (2007) 18712–18717.
[39] S. Thao, C.S. Chen, H. Zhu, J.C. Escalante-Semerena, N^{ε}-lysine acetylation of a bacterial transcription factor inhibits Its DNA-binding activity, PLoS ONE 5 (2010) e15123.
[40] M.D. Johnson, N.A. Burton, B. Gutierrez, K. Painter, P.A. Lund, RcsB is required for inducible acid resistance in *E. coli* and acts at *gadE* dependent and independent promoters, J. Bacteriol. (2011), doi:10.1128/JB.05040-11.
[41] K.A. Datsenko, B.L. Wanner, One-step inactivation of chromosomal genes in *Escherichia coli* K-12 using PCR products, Proc. Natl. Acad. Sci. USA 97 (2000) 6640–6645.