Hypochlorous acid (HOCl), the active ingredient of household bleach, functions as a powerful antimicrobial that is used not only in numerous industrial applications but also in mammalian host defence. Here we show that multicopy expression of \textit{cpdA}, encoding the cAMP phosphodiesterase, leads to a dramatically increased resistance of \textit{Escherichia coli} to HOCl stress as well as to the unrelated hydrogen peroxide (H$_2$O$_2$) stress. This general oxidative stress resistance is apparently caused by the CpdA-mediated decrease in cellular cAMP levels, which leads to the partial inactivation of the global transcriptional regulator cAMP receptor protein (CRP). Downregulation of CRP in turn causes the derepression of \textit{rpoS}, encoding the alternative sigma factor \(\sigma^S\), which activates the general stress response in \textit{E. coli}. We found that these highly oxidative stress-resistant cells have a substantially increased capacity to combat HOCl-mediated insults and to degrade reactive oxygen species. Mutational analysis revealed that the DNA-protecting protein Dps, the catalase KatE, and the exonuclease III XthA play the predominant roles in conferring the high resistance of \textit{rpoS}-overexpressing strains towards HOCl and H$_2$O$_2$ stress. Our results demonstrate the close regulatory interplay between cellular cAMP levels, \(\sigma^S\) activity and oxidative stress resistance in \textit{E. coli}.

**INTRODUCTION**

Challenge of bacteria with reactive oxygen species (ROS) such as superoxide, hydrogen peroxide (H$_2$O$_2$), hydroxyl radicals and hypochlorous acid (HOCl) causes a stress condition generally termed oxidative stress (Storz & Imlay, 1999). ROS arise endogenously as byproducts of respiration, and are often released by animals, plants and insects as a defence against microbial pathogens (Imlay, 2003; Apel & Hirt, 2004). HOCl is one of the most potent physiological antimicrobials, a property that arises from its ability to cause oxidative unfolding of cellular proteins (Winter \textit{et al.}, 2008). It is produced by neutrophils, which kill bacteria by ingesting them into phagosomes, where large amounts of HOCl are generated, and plays an important role in mucosal barrier epithelia, where HOCl apparently inhibits bacterial colonization (Ha \textit{et al.}, 2005; Winterbourn \textit{et al.}, 2006). Owing to their high reactivity, ROS cause oxidative damage to macromolecules, eventually leading to genome-wide mutations, protein inactivation and/or aggregation, and perturbations in membrane structure and function (reviewed by Imlay, 2003; Davies, 2005). Not surprisingly, a hallmark of the \textit{Escherichia coli} response to oxidative stress is the induction of genes encoding ROS scavenger proteins as well as proteins involved in DNA and protein repair (Zheng \textit{et al.}, 2001; Blanchard \textit{et al.}, 2007). In addition to the ROS-mediated activation of transcription factors such as OxyR and the concomitant induction of antioxidant genes, other proteins are activated by oxidative stress. One such example in \textit{E. coli} is the redox-regulated heat-shock protein Hsp33 (Jakob \textit{et al.}, 1999), whose function as molecular chaperone becomes activated upon oxidative stress (Graf \textit{et al.}, 2004; Ilbert \textit{et al.}, 2007; Winter \textit{et al.}, 2008). Active Hsp33 is an efficient chaperone holostate, protecting a large number of cellular proteins from stress-induced...
aggregation, which is pivotal for the survival of cells (Winter et al., 2005, 2008).

In this study, we set out to identify novel genes whose multicopy expression protects *E. coli* against HOCl-induced oxidative stress. We used a genomic expression library to screen for oxidative stress-resistant clones. In one clone, which conferred high resistance towards either HOCl or H$_2$O$_2$ stress, the observed resistance was found to be connected to decreased cAMP levels, which led to the partial inactivation of the cAMP receptor protein (CRP) and the accumulation of the general stress transcription factor RpoS (i.e. σ$^s$) in exponentially growing *E. coli* cells. We demonstrated that the high oxidative stress resistance is entirely mediated by σ$^s$, as the deletion of rpoS in these strains completely abolishes the oxidative stress resistance. Mutant studies revealed that while XthA and the σ$^s$-mediated overexpression of katE are responsible for the increased ROS-deteriorating capacity and H$_2$O$_2$ stress resistance of these strains, overexpression of dps appears to be most critical for their exquisite HOCl stress resistance.

**RESULTS AND DISCUSSION**

**Multicopy expression of cpdA confers resistance to oxidative stress**

HOCl is one of the most potent physiological antimicrobials. Despite the prominent role of HOCl, little is known about the bacterial response system(s) towards this potent oxidant. Recent experiments have revealed that expression of Hsp33 confers increased resistance upon *E. coli* cells towards HOCl treatment at both non-stress and elevated temperatures (Winter et al., 2005, 2008). This became especially evident when Hsp33 was overexpressed in the ΔrpoH strain BB7224, which lacks the alternative sigma factor σ$^s$ and is devoid of the general heat-shock response. In an attempt to identify other genes that, when expressed from a multicopy plasmid, are able to confer HOCl resistance to ΔrpoH cells, we used a genomic overexpression library and selected for clones that survived exposure to 6 mM HOCl in LB medium at 43 °C. We identified several clones, but one was particularly resistant to this oxidative heat treatment, and from this clone, the corresponding plasmid pJW22 was isolated. We transformed pJW22 into ΔrpoH and the corresponding wild-type (WT) strain MC4100 (KG27), and analysed their resistance on HOCl-containing LB plates at various temperatures. The presence of pJW22 resulted in an approximately 700-fold increase in viable titre for both strains (data not shown for ΔrpoH) compared with control cells (Fig. 1). This confirmed that the observed HOCl resistance in the original library clone was due to pJW22 and not due to a chromosomal mutation. It furthermore showed that pJW22 also conferred HOCl resistance at non-stress temperatures that was independent of the presence or absence of the heat-shock sigma factor σ$^s$.

Sequence analysis of pJW22 revealed the presence of a ~3 kb insert with two complete ORFs, yqiB and cpdA. Upon cloning of both genes separately into pUC18 and comparison of their HOCl resistance, it became obvious that only multicopy expression of cpdA conferred HOCl resistance. Importantly, only one of the two tested transformants expressing cpdA from the multicopy plasmid pKG2 (KG5) showed the same high resistance to HOCl treatment as pJW22 (Fig. 1). The second clone (KG4), however, was as sensitive to HOCl treatment as the WT (Fig. 1), supposedly due to a point mutation at base 281 in cpdA that fortuitously resulted in the replacement of Asp94 with Ala. Notably, Asn94 is one of the most highly conserved amino acids in CpdA, forming part of its predicted metal-binding site (Richter, 2002) and being essential for the catalytic activity of CpdA in *Mycobacterium tuberculosis* (Shenoy et al., 2005). These results strongly indicated that the catalytic activity of CpdA is responsible for the increased oxidative stress resistance observed in KG5.

**Multicopy expression of cpdA leads to partial inactivation of CRP**

The cellular concentration of cAMP is regulated by the adenylate cyclase CyaA, which synthesizes cAMP, and by

**METHODS**

**Bacterial strains and plasmids.** *E. coli* strains used in this study are listed in Supplementary Table S1. Detailed protocols on the generation of strains, plasmids and the genomic library are given in the supplementary material.

**Culture conditions.** Strains were cultivated in Luria–Bertani (LB) or M9 medium supplemented with appropriate antibiotics. Overnight cultures were diluted and cultivated at 30 or 37 °C to OD$_{600}$ 0.5 (LB) or OD$_{600}$ 0.3 (M9), unless otherwise indicated. For detailed information on the oxidative stress treatment, see supplementary material.

**Determination of intracellular cAMP concentration.** The concentration of cAMP was measured using the cAMP EIA kit (Cayman Chemical Company). A 200 μl volume of cells cultivated in M9 medium to OD$_{600}$ 0.6–0.7 was transferred into pre-heated tubes and boiled for 5 min. The lysate was centrifuged (1200 g, 5 min, 4 °C), and the supernatant was treated and assayed according to the manufacturer’s instructions. Samples were analysed in duplicate.

**Determination of σ$^s$ levels.** *E. coli* strains were cultivated in M9 or LB medium at 37 °C. Samples were removed during exponential or stationary growth and cell pellets were analysed by Western blotting with monoclonal antibodies to σ$^s$ (NeoClone Biotechnology International).

**Electron microscopy.** Scanning electron microscopy (SEM) and transmission electron microscopy (TEM) were performed on *E. coli* strains growing exponentially in M9 medium. For details, see supplementary material.

**Analysis of H$_2$O$_2$ in the cultivation medium.** A 500 μl volume of *E. coli* culture grown at 37 °C in M9 medium was diluted in 10 ml fresh M9 medium. Then, 0.5 mM H$_2$O$_2$ was added, and growth was continued for 2 h at 37 °C. Samples were removed before and during the stress, and the remaining H$_2$O$_2$ was determined using the FOX method (Wolff, 1994). For details, see supplementary material.
the phosphodiesterase CpdA, which hydrolysates cAMP. To investigate whether the pUC18-driven multicopy expression of \textit{cpdA} affects cellular cAMP levels, we determined the cAMP concentration in WT and KG5 strains. While the WT strain MC4100 had the expected cAMP concentration of $10.0 \pm 1.3$ pmol ml$^{-1}$ (Lengeler & Postma, 1999), multicopy expression of \textit{cpdA} led to a 10-fold reduction in cAMP levels ($1.0 \pm 0.1$ pmol ml$^{-1}$). This was similar to the findings of earlier reports, which show that multicopy expression of \textit{cpdA} decreases the relative cAMP levels to about 10% and significantly downregulates transcription of the \textit{lac} operon (Imamura et al., 1996). To investigate whether these reduced cAMP levels affect the activity of CRP, we tested the WT and KG5 strains for growth on alternative carbon sources. We found that multicopy expression of \textit{cpdA} led to a reduced ability of cells to use glycerol and lactose, strongly suggesting that CRP is at least partially inactivated (data not shown).

\textit{E. coli} cells lacking \textit{crp} (KG24) or \textit{cyaA} (FS16) are completely unable to grow on these carbon sources (data not shown; Shah & Peterkofsky, 1991; Botsford & Harman, 1992).

Strains lacking cAMP-CRP are highly resistant to oxidative stress

To investigate whether the increased HOCl resistance of the WT + \textit{cpdA} strain is directly due to altered cAMP levels or indirectly due to the inactivation of CRP, we decided to analyse and compare its oxidative stress resistance with the oxidative stress resistance of \textit{Δcrp} and \textit{ΔcyaA} strains. Although both strains lack a functional CRP system, \textit{Δcrp} has very high cAMP levels (437 $\pm$ 52 pmol ml$^{-1}$), while \textit{ΔcyaA} is unable to synthesize cAMP (Shah & Peterkofsky, 1991). As shown in Fig. 2(a), both strains were highly resistant to oxidative stress treatment and showed an almost 10 000-fold higher viable titre on LB + HOCl plates than the isogenic WT strain. Similar results were obtained when these strains were exposed to HOCl in liquid LB medium (data not shown). These strains were similarly resistant to \textit{H$_2$O$_2$} in either M9 medium (Fig. 3a and data not shown for \textit{ΔcyaA}) or LB medium (data not shown). A more than 1000-fold higher viable titre was observed for \textit{Δcrp} and \textit{ΔcyaA} strains after exposure to 6 mM \textit{H$_2$O$_2$} as compared with the WT. Although the WT + \textit{cpdA} strain was significantly more resistant to HOCl (Fig. 2a) and \textit{H$_2$O$_2$} (Fig. 3a) than WT \textit{E. coli}, it never reached the high oxidative stress resistance observed in \textit{Δcrp} or \textit{ΔcyaA} cells. This suggested that the residual amount of cAMP present in this strain is sufficient to confer partial CRP activity. Notably, WT and WT + \textit{cpdA} cells showed a very similar growth rate; we can therefore exclude the possibility that the higher oxidative stress resistance of WT + \textit{cpdA} cells results from an altered growth rate.

Our results demonstrated that cells lacking active cAMP-CRP are resistant to at least two different oxidative stressors, \textit{H$_2$O$_2$} and HOCl, both of which are effective...
Inactive cAMP-CRP confers oxidative stress resistance

strains are actually more sensitive to H₂O₂ treatment than the corresponding WT (Gonzalez-Flecha & Demple, 1997). These discrepancies cannot be imputed to differences in growth conditions because those authors used LB or M9 medium, in which we observe stress resistance of Δcrp and ΔcytA strains. To exclude the possibility that our results were allele- or strain background-specific, we also tested MG1655 and BW25113 strains lacking crp and found a similarly high increase in oxidative stress resistance (data not shown). At this time, we are unable to rationalize the difference between our observations and those of Gonzalez-Flecha & Demple (1997). It should be noted that although Δcrp and ΔcytA cells grow more slowly than WT cells, they have a similar number of cells per OD unit as WT cells, and all strains reach the same final OD in stationary phase (data not shown).

**Oxidative stress resistance in cAMP-CRP-deficient cells is due to increased σ^S levels**

What is the underlying mechanism of the oxidative stress resistance that we observe in cells lacking functional CRP? The global regulator CRP has been shown to positively affect the expression of the gene that encodes the oxidative stress transcription factor OxyR (Gonzalez-Flecha & Demple, 1997), and has been suggested to have an indirect, negative effect on the expression of rpoS, which encodes the alternative sigma factor σ^S that regulates the general stress response in *E. coli* (Hengge-Aronis, 2002). Recently, in *Vibrio vulnificus*, it has been shown that CRP binds directly to upstream regions of rpoS, resulting in the repression of rpoS expression (Lee et al., 2008). Because lack of oxyR induction in a Δcrp strain would decrease rather than increase the oxidative stress resistance of cells, we investigated whether the lack of rpoS repression could account for the increased oxidative stress resistance.

To test whether the partial inactivation of CRP in WT + cpdA cells is sufficient to increase the intracellular σ^S concentration during exponential growth to a degree that would explain the observed oxidative stress resistance, we compared the concentration of σ^S in exponentially growing WT, WT + cpdA and the corresponding Δcrp and ΔcytA strains. We found that the cellular concentration of σ^S in exponentially growing cells cultivated in either LB medium (Fig. 2b) or M9 medium (data not shown) was about three times higher in the WT + cpdA strain than in the corresponding WT strain. In cells lacking functional CRP, the σ^S levels were about 10-fold higher than in WT cells (Fig. 2b) and very similar to σ^S levels observed in stationary-phase *E. coli* cells (data not shown). Once the stationary growth phase was reached, however, the σ^S levels became very similar in all strains. These results suggest that partial or complete inactivation of CRP causes the derepression of rpoS transcription during exponential growth and leads to the accumulation of σ^S in exponentially growing cells. Similarly, increased σ^S levels have been observed in a cytA mutant (Lange & Hengge-Aronis, 1994), antimicrobials. Notably, WT + cpdA cells and cells lacking active cAMP-CRP were also more resistant to heat stress at 49 °C than WT cells (data not shown). Although it has been shown before that Δcrp and ΔcytA strains are resistant to stress conditions such as high temperature and gamma and UV radiation (Kumar, 1976), no increased resistance towards oxidative stress has been reported. On the contrary, Jenkins and co-workers have reported that a ΔcytA strain is as H₂O₂-sensitive as the WT (Jenkins et al., 1988), whereas others have found that Δcrp cytA or ΔcytA

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**Fig. 3.** Oxidative stress resistance is mediated by σ^S. (a) *E. coli* strains BB7222 (WT), WT + cpdA (KG5), ΔrpoS (JW367), ΔrpoS + cpdA (JW393), ΔoxyR (JW303), ΔoxyR + cpdA (JW308), Δcrp (KG24) and Δcrp rpoS (JW368) were cultivated at 37 °C in M9 medium. Plasmid-encoded expression of cpdA is indicated by ‘+’. Cultures were challenged with 6 mM H₂O₂ for 90 min, and samples were removed, serially diluted and spotted onto LB plates. The viability of non-stressed cells was taken as 100 %. The mean and SD of at least three independent experiments are shown. (b) *E. coli* strains BB7222 (WT), ΔrpoS (JW367), Δcrp (KG24) and ΔrpoS + rpoS (JW443) without arabinose (+rpoS) or with 0.4 % arabinose (+rpoS*) were cultivated and stressed for the indicated time points as described in (a).
which is as oxidative stress-resistant as the Δcrp strain (Fig. 2a). That the σS response might be responsible for the observed oxidative stress resistance in CRP-deficient cells correlates well with earlier findings that show that the high H2O2 resistance of E. coli cells in the stationary growth phase depends on increased rpoS expression (McCann et al., 1991; Lange & Hengge-Aronis, 1991b; Altuvia et al., 1994; Loewen & Hengge-Aronis, 1994). Our results suggest that cells with high σS levels induced by multicopy expression of cpdA, inactive CRP or growth in stationary phase (data not shown) are highly resistant to oxidative stress.

The increased oxidative stress resistance in cpdA-expressing strains is mediated by σS

The σS regulon exhibits an extensive regulatory overlap with the cAMP-CRP regulon and about 55% of all σS-controlled genes exhibit putative cAMP-CRP-binding sites in their 200 bp upstream regions (Weber et al., 2005). To test directly whether the oxidative stress resistance observed in WT + cpdA cells, which have partially inactive CRP, is indeed mediated by σS, we analysed the H2O2 sensitivity of ΔrpoS cells in the presence or absence of the cpdA plasmid pKG2. ΔrpoS cells showed a very high sensitivity to oxidative stress treatment, which was rescued by rpoS expression (pJW25, Fig. 3b) but, more importantly, no longer by pKG2 (Fig. 3a). Similarly, we found that Δcrp rpoS cells were as sensitive to oxidative stress as the ΔrpoS strain (Fig. 3a). This result clearly demonstrated that the CRP-mediated upregulation of σS and the concomitant increase in σS-dependent gene expression are responsible for the observed oxidative stress resistance in our mutant strains. This acquired oxidative stress resistance was independent of the peroxide-specific transcription factor OxyR, because multicopy expression of cpdA was fully able to restore the oxidative stress resistance of the ΔoxyR strain to WT levels (Fig. 3a). Although our results are contrary to the study by Gonzalez-Flecha and coworkers, who showed that overexpression of rpoS increases the H2O2 sensitivity of E. coli (Gonzalez-Flecha & Demple, 1997), they are in line with other studies that show that high levels of rpoS in stationary phase confer oxidative stress resistance (see above). Furthermore, it has been suggested that σS also contributes to cellular HOCl resistance (Dukan & Touati, 1996).

E. coli cells with high σS levels have thicker cell walls

HOCl and H2O2 are two chemically distinct oxidants. While HOCl is highly reactive and rapidly and largely non-selectively reacts with all biological macromolecules (Hawkins et al., 2003), H2O2 shows relatively slow reaction rates, even with preferred targets such as the sulfur-containing amino acids cysteine and methionine (Imlay, 2003). In response to HOCl, bacteria appear to induce the heat-shock and SoxR response (Dukan et al., 1996), while H2O2 induces the OxyR response. These considerations raised the question of how the σS response can mediate the high oxidative stress resistance towards two seemingly unrelated oxidants. Because changes in the cell surface, shape and cell wall are typical properties of stress-resistant stationary-phase E. coli cells (Lange & Hengge-Aronis, 1991b; Santos et al., 2002), we compared the morphology of our WT, the oxidative stress-sensitive (ΔrpoS, Δcrp rpoS) and the stress-resistant (Δcrp, WT + cpdA) E. coli strains during exponential growth by SEM and TEM (Fig. 4; Supplementary Table S2). While the cell surface of all strains looked similar, the individual strains differed substantially in their cell shape (Fig. 4, left panels). E. coli cells with increased σS were substantially shorter but also wider than WT cells, while stress-sensitive ΔrpoS cells were elongated with increased cell volume, which is consistent with earlier observations (Lange & Hengge-Aronis, 1991a). Surprisingly, however, the stress-sensitive Δcrp rpoS cells had a cell shape similar to that of the oxidative stress-resistant Δcrp cells. This suggests that the observed changes in cell shape are likely to be mediated by CRP and do not directly correlate with σS levels or with the oxidative stress resistance of E. coli cells. To analyse potential morphological changes in more detail, we analysed ultrathin sections of exponentially growing, embedded E. coli cells by TEM (Fig. 4, right panels, Supplementary Table S2). We focused on the cell wall (dark contrast) and the adjacent periplasm (light contrast) and estimated their dimensions. In WT E. coli, the cell wall and periplasm had a combined volume of about 5–10% of the total cell volume. This was very similar in ΔrpoS (5–8%) and Δcrp rpoS (3–11%) cells, suggesting that decreased resistance to oxidative stress does not correlate to changes in cell wall and periplasmic dimensions. In contrast, however, we found that increased resistance to oxidative stress was directly correlated with increased cell wall thickness. WT + cpdA cells had a twofold larger combined cell wall/periplasmic volume (10–14% of the total cell volume), while the Δcrp strain showed an even larger volume (12–37%) that was similar to that of ΔcyA cells and WT cells in stationary phase (Supplementary Fig. S1). Neither the cell volume (Supplementary Table S2) nor the total protein amount (not shown) was significantly altered in these cells. These results suggest that the observed morphological changes correlate with increased σS levels and an increased oxidative stress resistance.

Increased peroxide stress resistance due to rapid intracellular ROS detoxification

The morphological changes that we observed correlated well with increased RpoS levels and oxidative stress resistance and suggested that thicker cell walls provide more efficient physical barriers against ROS and prevent ROS from effectively penetrating cells. Because correlations do not necessarily reflect a causal relationship, we analysed the ability of our cells to degrade exogenously added H2O2 (Fig. 5). This is indicative of the amount of H2O2 diffusing into cells and being degraded by cytoplasmic hydroperoxides such as catalase. We found that WT, ΔrpoS and Δcrp
rpoS strains degraded about 30% of the initial H2O2 within the first 30 min and 45–60% 2 h after addition of the oxidant. The stress-resistant Δcrp cells, in contrast, removed more than 50% of H2O2 within the first 30 min and nearly all of it within 2 h of incubation. This presumably explains why Δcrp cells, in contrast to WT and rpoS mutant cells, can resume growth after this point (data not shown). The high H2O2 degradation capacity of the Δcrp strain appears to be largely mediated by the catalase KatE, because a Δcrp katE strain degraded H2O2 significantly more slowly, and the level of H2O2 did not decrease to the very low level produced by the Δcrp cells (data not shown). Multicopy expression of rpoS increased the H2O2 degradation capacity (Fig. 5), although even maximal σ5 induction did not confer the same high H2O2 degradation activity observed in Δcrp cells. This result is consistent with our earlier observation, in which maximal σ5 levels were not able to confer the high oxidative stress resistance observed in Δcrp strains. It suggests either that not all of the expressed RpoS is active or that CRP negatively controls additional factor(s) that increase the oxidative stress resistance of bacteria in an RpoS-independent manner (see Fig. 3b). In summary, our results suggest that the oxidative stress resistance observed in Δcrp strains is not, at least primarily, due to thicker cell walls that could serve as an ROS barrier. This is in contrast to the case in Vibrio cholerae, which upon shifting from its normal translucent to rugose colony morphology in response to nutrient starvation, is found to produce exopolysaccharide material on its surface that significantly promotes H2O2 resistance.

**Fig. 4.** Electron microscopic images of E. coli WT and mutant cells. Cultures of BB7222 (WT), WT + cpdA (KG5), Δcrp (KG24), ΔrpoS (JW367) and Δcrp ΔrpoS (JW368) were grown in M9 medium at 37°C to OD600 0.3. Whole cells were analysed by SEM (left panels) and ultrathin sections of cells were analysed by TEM (right panels).
H_{2}O_{2} remaining in media (\%)

![Figure 5](image)

**Fig. 5.** Effect of \(\sigma^{S}\) on ROS degradation capacity. *E. coli* strains BB7222 (WT), \(\Delta crp\) (KG24), \(\Delta rpoS\) (JW367), \(\Delta crp\ rpoS\) (JW368) and \(\Delta rpoS+rpoS\) (JW443) supplemented with 0, 0.2, 0.4 or 1 % arabinose were cultivated at 37 \(^{\circ}\)C in M9 medium. The H_{2}O_{2} degradation capacity of WT and mutant cells was determined by measuring the residual amount of H_{2}O_{2} in cultures supplemented with 0.5 mM H_{2}O_{2} after 30 min (white bars) or 2 h (striped bars). The mean and SD of at least three independent experiments are presented.

(Wai et al., 1998). Our results, on the other hand, suggest that the resistance observed in \(\Delta crp\) strains is mediated by increased detoxification of ROS.

### Which \(\sigma^{S}\)-regulated genes are involved in oxidative stress resistance?

About 150 genes are considered to be the core genes of the \(\sigma^{S}\) regulon. Their expression is induced under salt stress, pH stress and entry into stationary phase (Weber et al., 2005). To identify \(\sigma^{S}\)-regulated genes that confer oxidative stress resistance in \(\Delta crp\) cells, we generated a series of gene deletions in WT and \(\Delta crp\) cells. We focused primarily on those \(\sigma^{S}\)-dependent genes that are involved in cell morphology (bolA) or are suggested to be involved in oxidative stress resistance (katE, dps, xthA, osmC). Both the catalase KatE and the DNA-binding protein Dps play a role in the H_{2}O_{2} resistance of stationary-phase growing or starving *E. coli* cells (Almiron et al., 1992; Dukan & Touati, 1996; Nair & Finkel, 2004). In addition, \(dps\) expression is also under OxyR control and is massively increased in exponentially growing *E. coli* cells upon H_{2}O_{2}-stress treatment (Altuvia et al., 1994; Zheng et al., 2001). xthA does not seem to be a member of the RpoS regulon (Weber et al., 2005); however, it has been shown to be upregulated in a OxyR suppressor strain that has increased \(\sigma^{S}\) levels. Such increased \(\sigma^{S}\) levels are able to reverse the H_{2}O_{2} hypersensitivity of OxyR mutants (Ivanova et al., 1997). OsmC, which is primarily important under salt stress, is able to degrade H_{2}O_{2} *in vitro* (Lesniak et al., 2003; Weber et al., 2006), and starving ΔosmC cells seem more H_{2}O_{2}-sensitive than the corresponding WT (Conter et al., 2001). bolA is induced upon oxidative stress and is a morphogene whose overexpression causes the round cell morphology observed in stationary-phase growing cells and presumably in our \(\Delta crp\) cells (see Fig. 4) (Aldea et al., 1988; Santos et al., 1999).

To determine whether any of these proteins contribute to the increased oxidative stress resistance of \(\Delta crp\) strains, we deleted the individual genes in both the WT and \(\Delta crp\) backgrounds and analysed their viability in response to H_{2}O_{2} (Fig. 6a) or HOCl (Fig. 6b) treatment. Deletion of osmC or bolA did not significantly alter the viability of WT or \(\Delta crp\) cells during either stress treatment, confirming our previous observations that changes in cell morphology do not contribute to increased oxidative stress resistance. In contrast, KatE protects \(\Delta crp\) but not WT cells against H_{2}O_{2} stress only (Fig. 6). These results agreed with the facts that the *katE* gene is solely RpoS-controlled and that \(\Delta crp\) cells have a higher ROS-detoxifying activity than WT cells (Fig. 5). At the same time, however, this result excluded the possibility that RpoS-mediated *katE* expression is responsible for the observed HOCl-stress resistance of \(\Delta crp\) strains. This is in line with earlier data showing that a *katE* deletion mutant survives HOCl stress with the same viability as the corresponding WT strain (Dukan & Touati, 1996). The exonuclease III XthA is clearly involved in mediating H_{2}O_{2} resistance but not resistance towards HOCl (Fig. 6). Deletion of *xthA* renders WT and \(\Delta crp\) cells sensitive to H_{2}O_{2} stress, and the viability of the mutant strains is reduced up to 100-fold compared with the respective controls (Fig. 6a). This is in line with the observation that increased \(\sigma^{S}\) levels and increased *xthA* transcription in a suppressor mutant reverse the H_{2}O_{2}-hypersensitivity of a OxyR strain (Ivanova et al., 1997). The protective effect of XthA, however, is restricted to H_{2}O_{2} stress, as deletion of *xthA* does not alter the HOCl resistance of *E. coli* cells (Fig. 6b). In contrast, deletion of the *dps* gene significantly affected both H_{2}O_{2} and HOCl stress resistance in WT and \(\Delta crp\) strains (Fig. 6a, b). That Dps confers oxidative stress resistance in exponentially growing WT cells indicates that the amount of \(\sigma^{S}\) made during exponential growth is high enough to promote constitutive *dps* expression. A similar observation has been made in *E. coli* O157:H7, in which Dps levels during exponential growth are sufficient to confer some of the observed acid tolerance (Jeong et al., 2006). The viability of the \(\Delta crp\) *dps* strain upon exposure to H_{2}O_{2} or HOCl was at least two to three orders of magnitude lower than that of the isogenic \(\Delta crp\) strain (Fig. 6), indicating that Dps indeed plays a central role in the general defence of *E. coli* cells against oxidative stress. Yet \(\Delta crp\) *dps* cells are still several orders of magnitude more resistant to HOCl than WT cells, indicating that other, yet to be identified factors serve to protect *E. coli* from HOCl-mediated killing. Considering that HOCl causes oxidative unfolding and aggregation of proteins (Winter et al., 2008), an unfolded protein response that upregulates chaperones...
and proteases would be ideally suited to alleviate the stress caused by HOCl. The two known functions of Dps during H2O2 stress are the oxidation of Dps-bound iron, which effectively prevents the formation of the highly toxic hydroxyl radicals, and the formation of protective Dps–DNA co-crystals (Wolf et al., 1999). How Dps protects against HOCl stress is not yet known. We found that E. coli cells lacking dps (Ddps and Dcrp dps) undergo a significantly higher extent of spontaneous and HOCl-induced mutagenesis than the respective isogenic controls (data not shown), suggesting that the DNA-protective function of Dps also extends to HOCl stress. Our results demonstrate that the RpoS-mediated upregulation of both katE and dps is predominantly responsible for the acquired oxidative stress resistance observed in Δcrp strains and in strains with increased rpoS levels in general.

ACKNOWLEDGEMENTS

We thank Drs Fred Blattner, University of Wisconsin, Madison, and Bernd Bukau, Zentrum für Molekulare Biologie der Universität Heidelberg, for strains, and Drs Jim Bardwell, Janine Maddock and Robert Bender, and members of the Jakob, Bardwell and Buchner laboratories, for discussions. Furthermore, we thank Ryan Frisch for technical assistance, Drs Ken Balazovich and Sonja Bardy as well as Bettina Richter and Christoph Kaiser for assistance with electron microscopy, and Dr Sevil Weinkauf for the use of the electron microscopes. This work was supported by National Institute of Health Grant GM065318 and the Burroughs Wellcome Fund Career Award (to U. J.), and by the EliteNetzwerk Bayern (to K. M. G.), the Leopoldina Gesellschaft Deutscher Naturforscher, and the Emmy-Noether program of the Deutsche Forschungsgemeinschaft (DFG) (to J. W.).

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Fig. 6. Effect of the deletion of selected genes on the H2O2 and HOCl stress resistance of WT and Δcrp strains. (a) E. coli strains BB7222 (WT), ΔkatE (JW437), Δdps (JW404), ΔxthA (KMG197), ΔosmC (JW409), ΔbolA (JW320), Δcrp (KG24), Δcrp katE (JW330), Δcrp dps (JW416), Δcrp xthA (KMG199), Δcrp osmC (JW422) and Δcrp bolA (JW323) were cultivated at 37 °C in M9 medium. The viability after stress with 6 mM H2O2 for 90 min was determined as described in the legend to Fig. 3(a). (b) Strains as in (a) were cultivated in LB medium at 37 °C. The viability was analysed by preparing serial dilutions of cultures and spotting them onto LB plates containing 6 mM HOCl (WT, ΔkatE, Δdps, ΔxthA, ΔosmC, ΔbolA) or 7 mM HOCl (Δcrp, Δcrp katE, Δcrp dps, Δcrp xthA, Δcrp osmC, Δcrp bolA). The viability of non-stressed cells was taken as 100 %. The means and sds of up to five individual experiments are shown.


