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MINI-REVIEW

Revisiting long-chain fatty acid metabolism in *Escherichia coli*: integration with stress responses

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Abstract

Long-chain fatty acids (LCFAs) are a tremendous source of metabolic energy, an essential component of membranes, and important effector molecules that regulate a myriad of cellular processes. As an energy-rich nutrient source, the role of LCFAs in promoting bacterial survival and infectivity is well appreciated. LCFA degradation generates a large number of reduced cofactors that may confer redox stress; therefore, it is imperative to understand how bacteria deal with this paradoxical situation. Although the LCFA utilization pathway has been studied in great detail, especially in *Escherichia coli*, where the earliest studies date back to the 1960s, the interconnection of LCFA degradation with bacterial stress responses remained largely unexplored. Recent work in *E. coli* shows that LCFA degradation induces oxidative stress and also impedes oxidative protein folding. Importantly, both issues arise due to the insufficiency of ubiquinone, a lipid-soluble electron carrier in the electron transport chain. However, to maintain redox homeostasis, bacteria induce sophisticated cellular responses. Here, we review these findings in light of our current knowledge of the LCFA metabolic pathway, metabolism-induced oxidative stress, the process of oxidative protein folding, and stress combat mechanisms. We discuss probable mechanisms for the activation of defense players during LCFA metabolism and the likely feedback imparted by them. We suggest that besides defending against intrinsic stresses, LCFA-mediated upregulation of stress response pathways primes bacteria to adapt to harsh external environments. Collectively, the interplay between LCFA metabolism and stress responses is likely an important factor that underlies the success of LCFA-utilizing bacteria in the host.

Keywords β-oxidation · Cpx · DsbA · DsbB · Envelope stress response · Reactive oxygen species

Introduction

Long-chain fatty acids (LCFAs) are amphiphilic molecules composed of a linear aliphatic chain of 12–20 carbon atoms and a terminal carboxyl group. Several bacteria acquire LCFAs from host tissues, which essentially have three fates inside the bacterial cell: degradation via β-oxidation, incorporation into membrane phospholipids, and recognition as signaling molecules. Thus, depending on their fate, LCFAs can provide metabolic energy, remodel bacterial membrane, and govern bacterial response to the environment. The effect of LCFAs on bacterial processes such as virulence, biofilm formation, and motility via their incorporation into the membrane and as signaling molecules has been reviewed recently (Kumar et al. 2020). Further, studies have associated membrane phospholipid composition with stress. In the bacterial strains where the ratio of unsaturated to saturated LCFAs in membrane phospholipids was altered due to a mutation in the enzyme involved in membrane incorporation of LCFAs or varied by either overproducing free fatty acids or overexpressing a fatty acid biosynthesis enzyme, the unsaturated LCFA content in membranes was found to be directly correlated with oxidative and membrane stress (Lennen et al. 2011; Oberg et al. 2013; Pradenas et al. 2012).

Studies have suggested that LCFAs also induce stress in bacteria when used as a nutrient source (Doi et al. 2014; Rodriguez et al. 2014). Several bacteria obtain metabolic energy from host-derived LCFAs, which contributes to their survival and virulence. For example, the LCFA degradation enzymes are induced in *Mycobacterium tuberculosis*

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and *Pseudomonas aeruginosa* during lung infection (Pan et al. 2020; Schnappinger et al. 2003; Son et al. 2007); the LCFA degradation enzymes are upregulated in *Salmonella* Typhimurium during infection, which likely contributes to the metabolism of pro-inflammatory host LCFAs and thereby suppression of the innate immune response (Mahan et al. 1995; Spector et al. 1999); in *Vibrio cholerae*, cholera toxin-dependent remodeling of the host metabolism causes lipolysis in target cells, accumulating LCFAs in the intestinal lumen, which are ultimately used by the pathogen for its enhanced growth (Rivera-Chavez and Mekalanos 2019); and mutants of *M. tuberculosis*, *P. aeruginosa* and *S.* Typhimurium defective in LCFA utilization exhibit reduced virulence (Fang et al. 2005; Kang et al. 2010; McKinney et al. 2000; Munoz-Elias and McKinney 2005). The widespread use of LCFAs as a nutrient source warrants a detailed understanding of the mechanisms by which LCFA utilization induces stress and the cellular responses employed by bacteria to mitigate them. Recent studies have investigated this issue primarily in *Escherichia coli* (Agrawal et al. 2017; Jaswal et al. 2020). In this review, we first describe the LCFA utilization pathway in *E. coli* and then discuss the recently uncovered association of LCFA degradation with stress response mechanisms.

LCFA degradation pathway in *E. coli*

The LCFA utilization pathway has been extensively studied in *E. coli*, which can grow on this carbon source, both under aerobic and anaerobic conditions. LCFA metabolism is carried out by Fad (fatty acid degradation) proteins, which transport and activate LCFAs, and further, degrade these to acetyl-CoA via β-oxidation (Fig. 1). Briefly, the exogenous LCFAs are transported across the outer membrane by a β-barrel outer membrane protein, FadL. During aerobic metabolism, LCFAs are extracted from the inner membrane and activated to acyl-CoA thioesters by the inner membrane-associated acyl-CoA synthetase, FadD. Acyl-CoAs are further degraded in the cytoplasm via the various activities of β-oxidation enzymes FadE, FadB, and FadA. In each round of β-oxidation, two carbon atoms are released as acetyl-CoA,

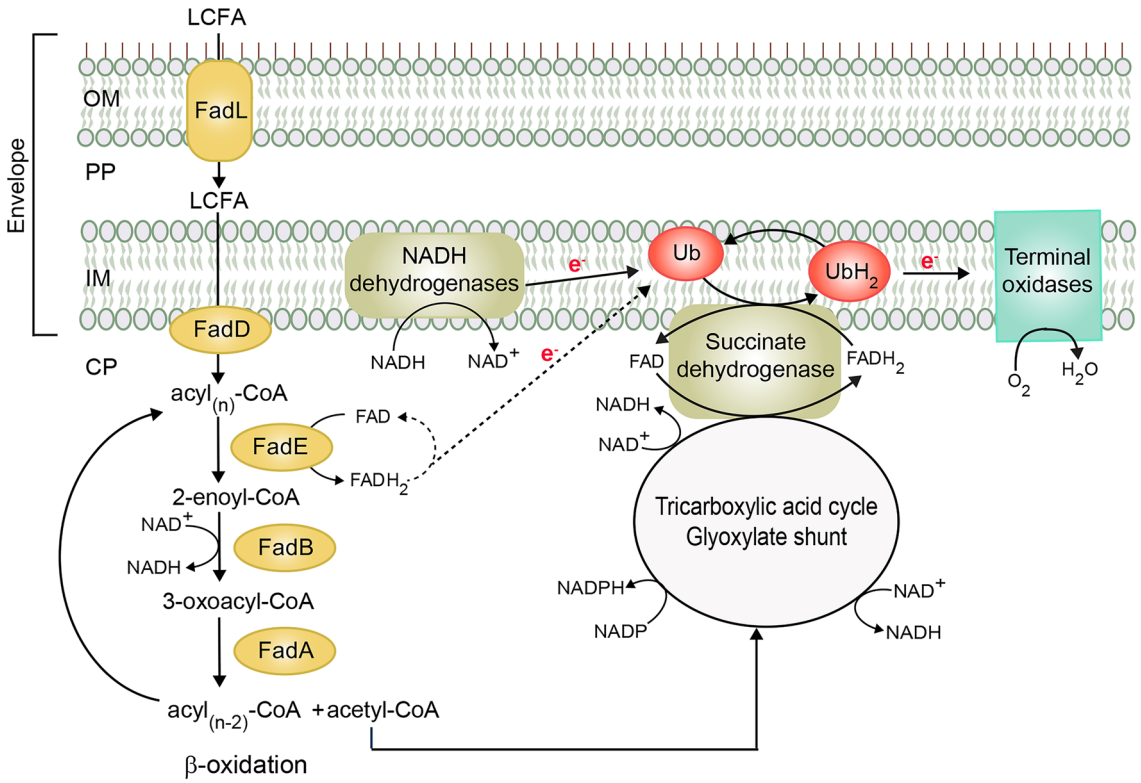

Fig. 1 The aerobic LCFA utilization pathway in *E. coli*. Exogenously provided LCFAs are transported across the outer membrane by FadL. LCFAs are extracted from the inner membrane and activated to acyl-CoA by FadD. Acyl-CoAs are degraded via the β-oxidation enzymes FadE, FadB, and FadA, to acetyl-CoA, which is further metabolized in the TCA and glyoxylate cycles. NADH and $FADH_2$ generated in β-oxidation and TCA cycle are oxidized in the ETC by respiratory dehydrogenases, and the electrons are transferred to ubiquinone. Ubiquinol further gives electrons to the terminal oxidases. Arrows with e^- labels indicate the direction of electron flow. The dotted arrow denotes that the players involved in FadE oxidation and transfer of electrons from FadE to the ETC are not known. *CP* cytoplasm, *IM* inner membrane, *PP* periplasm, *OM* outer membrane, *Ub* ubiquinone, UbH_2 ubiquinol

and the shortened acyl-CoA re-enters the degradation cycle. Acetyl-CoA enters the tricarboxylic acid (TCA) and glyoxylate cycles to generate metabolic precursors for growth (Fig. 1). LCFA metabolism is mainly regulated at a transcriptional level by three systems: (i) negative regulation by the transcriptional regulator, FadR, whose repression is relieved by binding of acyl-CoA, (ii) negative regulation by the ArcA–ArcB (anoxic redox control) two-component system, and (iii) positive regulation by the cAMP-CRP (cyclic AMP receptor protein-cyclic AMP) complex [reviewed in Clark and Cronan (2005), Cronan and Laporte (2006)].

Unlike glucose, a fermentable carbon source, which generates energy both in glycolysis and in the electron transport chain (ETC), LCFAs being non-fermentable carbon sources generate energy in the ETC (Berger 1973; Campbell et al. 2003; Clark and Cronan 2005; Romeo and Snoep 2005). The ETC components are present in the inner membrane of *E. coli* (Fig. 1). During aerobic metabolism of LCFAs, the reduced cofactors, NADH and $FADH_2$, generated in β-oxidation and TCA cycle, are oxidized at the ETC by NADH dehydrogenases and succinate dehydrogenase, respectively, and the electrons are transferred to ubiquinone, a lipid-soluble electron carrier. Ubiquinol, the reduced form of ubiquinone, in turn, donates electrons to the terminal oxidases, which finally transfer electrons to molecular oxygen (O_2) (Aussel et al. 2014b; Unden et al. 2014). The acyl-CoA dehydrogenase, FadE, which catalyzes the first step of β-oxidation, i.e., the conversion of acyl-CoA to enoyl-CoA together with the reduction of FAD to $FADH_2$, has also been hypothesized to re-oxidize $FADH_2$ by transferring electrons to the ETC (Fig. 1) (Campbell and Cronan 2002). During electron transfer through ETC, proton motive force is generated, which is then used by ATP synthase to drive ATP synthesis (Unden et al. 2014).

LCFA metabolism and stress responses

Since LCFA degradation generates a large number of reduced cofactors (Fig. 1), its utilization may confer redox stress in bacteria. Studies in a laboratory strain of *E. coli*, summarized below, showed that LCFA utilization generates elevated levels of reactive oxygen species (ROS) and causes problems in oxidative protein folding; however, bacteria induce cellular responses to counteract the detrimental effect of these stresses (Agrawal et al. 2017; Jaswal et al. 2020).

LCFA metabolism-associated oxidative stress and combat mechanisms

During metabolism, ROS are formed as an inevitable consequence of redox reactions. The ROS molecules, superoxide (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radical ($^{\cdot}OH$), are produced by mechanisms that include electron leakage during oxidation-reduction cycles of ETC promoting the adventitious collision of free electrons with O_2, extraction of electrons from metal centers of several metabolic enzymes by O_2, and auto-oxidation of flavoproteins (Imlay 2003, 2013; Søballe and Poole 2000). *E. coli* grown aerobically in LCFAs generates higher levels of ROS compared to cells cultured in fermentable (glucose) or other non-fermentable (acetate and succinate) carbon sources (Agrawal et al. 2017; Doi et al. 2014). The *fad* mutants defective in different steps of LCFA transport and degradation are unable to produce ROS validating LCFA utilization as the reason for LCFA-induced oxidative stress (Agrawal et al. 2017). Importantly, the high $NADH/NAD^+$ and $FADH_2$/FAD ratios during LCFA metabolism increase electron flow in the ETC (Fig. 1) (Jaswal et al. 2020); this likely increases electron leakage and auto-oxidation of respiratory dehydrogenases. A predominant source of ROS during LCFA metabolism could be the flavoprotein involved in β-oxidation, i.e., the acyl-CoA dehydrogenase FadE. The auto-oxidation of reduced flavin bound to FadE might generate ROS (Agrawal et al. 2017).

The imbalance in ROS production and antioxidants leads to oxidative stress. The highly reactive ROS molecules oxidize macromolecular components resulting in DNA damage, lipid peroxidation, and disassembly of iron-sulfur clusters and formation of undesired disulfide bonds in proteins. For decades, the enzymes, catalases, peroxidases, and superoxide dismutases have been known as the major oxidative stress combat players in *E. coli* [reviewed in Chiang and Schellhorn (2012), Farr and Kogoma (1991), Imlay (2013)]. On the contrary, the role of ubiquinone as an antioxidant remained underappreciated.

In bacteria, the antioxidant function of ubiquinone was first suggested in a study by Søballe and Poole (2000), where an *E. coli* strain defective in ubiquinone biosynthesis was shown to exhibit several oxidative stress phenotypes in LB medium. However, the physiological condition under which ubiquinone plays a more prominent role in comparison to other oxidative stress response players had not been investigated. We identified ubiquinone as a key antioxidant during LCFA metabolism (Agrawal et al. 2017). The first hint came from the comparative analysis of the datasets from high-throughput genetic screens of the single-gene deletion library of *E. coli* on various carbon sources, which included non-fermentable carbon sources, acetate, succinate, and oleate (an LCFA; C18:1 cis-9). This large-scale analysis revealed that the requirement of aerobic ETC components for growth is inversely correlated with the ATP yield of non-fermentable carbon sources; their requirement is maximal in acetate, which has the poorest net ATP yield. However, the requirement of ubiquinone does not follow this trend; it is maximally

required for growth in LCFAs. Following observations revealed that the increased requirement of ubiquinone in LCFAs is to mitigate oxidative stress: (i) the growth defect of ubiquinone biosynthesis mutants in oleate is partially recovered by chemical antioxidants, glutathione and thiourea, (ii) the exogenous supplementation of ubiquinone decreases ROS production in oleate-grown cells, and (iii) oleate utilization generates ROS and also results in ubiquinone accumulation; thus a feedback loop likely prevents excessive ROS formation during LCFA metabolism. Further, as long as ubiquinone is present in LCFA-grown cells, it does not allow ROS to build-up, thereby reducing dependence on other oxidative stress response players: (i) in oleate-utilizing cells, ROS levels increase in strains defective in ubiquinone biosynthesis but not in strains deleted for other oxidative stress combat players, and (ii) during oleate metabolism, enzymatic scavengers are induced only in a ubiquinone biosynthesis mutant. Collectively, our study established ubiquinone as the cell's primary defense against LCFA-mediated oxidative stress (Agrawal et al. 2017).

There are at least two possible mechanisms by which ubiquinone might combat LCFA-induced ROS. Considering its electron shuttling role in the ETC, it is likely that the increased levels of ubiquinone in LCFA-utilizing cells enable the rapid transfer of a large flow of electrons derived from LCFA metabolism, decreasing the residence time of electrons at the site of ROS formation (Agrawal et al. 2017). Further, based on a previous finding that the terminal oxidase, Cyd, exhibits quinol peroxidase activity in vitro (Al-Attar et al. 2016), we can speculate that the increased accumulation of ubiquinol (reduced form of ubiquinone) during LCFA metabolism promotes the peroxidase activity of Cyd to scavenge ROS (Agrawal et al. 2017).

Besides upregulating antioxidant defense mechanisms to mitigate elevated ROS produced due to high NADH/NAD^+ and $FADH_2$/FAD ratios, it is plausible that bacteria induce anabolic pathways as a reductive sink to restore redox balance. In fact, the global transcriptome of *M. tuberculosis* cultured in a medium supplemented with a mixture of even-length LCFAs showed overexpression of WhiB3 and DosR, the two heme sensor proteins involved in maintaining intracellular redox balance, and of several genes involved in complex lipid biosynthesis, a process that consumes reduced cofactors (Rodriguez et al. 2014). Notably, the comparative analysis of the high-throughput genetic screens of the single-gene deletion library of *E. coli* on different carbon sources also revealed maximal enrichment of the anabolic pathway, gluconeogenesis, in LCFAs (Agrawal et al. 2017).

LCFA metabolism impedes oxidative protein folding and activates envelope stress response to restore homeostasis

Disulfide bond formation, an oxidative process that creates a covalent bond between the sulfur atoms of two cysteine residues, is required for the maturation and stability of many extracytoplasmic proteins in all domains of life. In Gram-negative bacteria, this process takes place in the oxidizing environment of the periplasm, an aqueous space surrounded by the outer and inner-membrane layers of the envelope. In *E. coli*, a periplasmic oxidoreductase, DsbA, forms disulfide bonds in substrate proteins. DsbB, an inner-membrane disulfide oxidoreductase, re-oxidizes DsbA and transfers electrons to quinones, ubiquinone and menaquinone, during

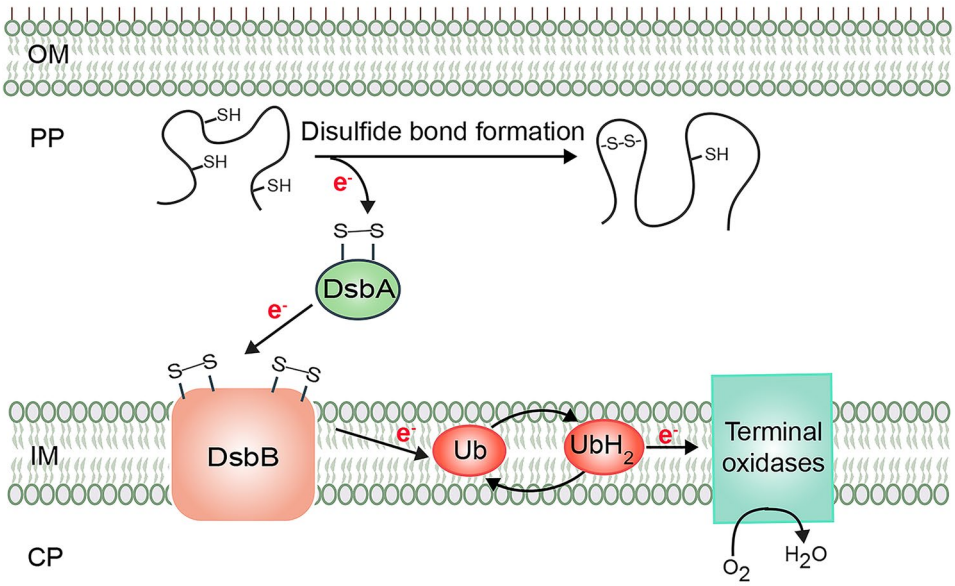

Fig. 2 The aerobic disulfide bond formation pathway in *E. coli*. DsbA oxidizes substrate proteins in the periplasm and becomes reduced. DsbB re-oxidizes DsbA by transferring electrons to ubiquinone. Ubiquinol further gives electrons to the terminal oxidases. Arrows with e^- labels denote the direction of electron flow. *CP* cytoplasm, *IM* inner membrane, *PP* periplasm, *OM* outer membrane, *Ub* ubiquinone, UbH_2 ubiquinol

aerobic and anaerobic metabolism, respectively (Fig. 2) (Landeta et al. 2018; Manta et al. 2019). The role of ETC in oxidative protein folding in *E. coli* has been demonstrated in several studies: (i) DsbA accumulates in a reduced form in mutants with a defective respiratory chain, i.e., in mutants defective in either heme or quinone biosynthesis (Kobayashi et al. 1997), (ii) disulfide bond formation is compromised in *E. coli* grown in a purely fermentative manner, a condition where the ETC is non-operational (Bader et al. 1999), (iii) in vitro reconstitution of the oxidative protein folding system showed the involvement of ubiquinone and terminal oxidases in re-oxidizing DsbA–DsbB (Bader et al. 1999), and (iv) mutants defective in ubiquinone biosynthesis exhibit thiol hypersensitivity (Zeng et al. 1998), a phenotype shared by *dsb* mutants (Bardwell et al. 1991, 1993; Missiakas et al. 1993).

An earlier study in *E. coli*, grown aerobically in glucose, reported ubiquinone to be present in ~ 15- to 20-fold excess over other ETC components (Cox et al. 1970). However, our observation that exogenous supplementation of ubiquinone decreases ROS production in oleate-grown cells strongly suggested ubiquinone to be limiting for its electron transfer function during LCFA metabolism (Agrawal et al. 2017). Given the convergence of metabolism and disulfide bond formation in the ETC, we investigated whether ubiquinone is also insufficient for oxidative protein folding in LCFA-utilizing cells (Jaswal et al. 2020). The various phenotypes exhibited by oleate-grown cells, i.e., decrease in the activity of alkaline phosphatase (a DsbA substrate), hypersensitivity to thiol agents, sensitivity to cadmium (binds with free thiols of proteins), and accumulation of the reduced form of DsbA and its substrate, DegP, convincingly established that disulfide bond formation is hampered during LCFA metabolism. Importantly, these hallmarks are prevented when ubiquinone is exogenously provided to LCFA-grown cells (Jaswal et al. 2020).

In *E. coli*, ~ 300 extracytoplasmic proteins are predicted to have disulfide bonds (Dutton et al. 2008). Of these, more than two dozen proteins involved in diverse biological processes are reported to be dependent on DsbA for correct folding (Delhaye et al. 2019; Kadokura et al. 2004; Manta et al. 2019). Thus, to ensure cellular homeostasis, it is important for bacteria to monitor the envelope redox status and mount an appropriate response when disturbances occur. During LCFA metabolism, DsbA accumulates in its reduced form only transiently (Jaswal et al. 2020), which suggested that defense mechanisms are upregulated to deal with the hypo-oxidizing environment of the envelope. Of the five dedicated envelope stress response (ESR) pathways (Bae, Cpx, Psp, Rcs, and σ^E), which sense damage in the envelope and change the transcriptome to mitigate stress (Mitchell and Silhavy 2019), we identified Cpx to be the major ESR system activated by LCFAs (Jaswal et al. 2020). Cpx is a two-component system comprised of an inner-membrane sensor histidine kinase, CpxA, and a cytoplasmic response regulator, CpxR. In the presence of envelope stress signals, CpxA autophosphorylates and transfers its phosphoryl group to CpxR, which then directs the transcription of its regulon members involved in combating stress (Grabowicz and Silhavy 2017; Raivio 2014; Raivio and Silhavy 1997). Importantly, Cpx induction in LCFA-grown cells is partially downregulated upon exogenous supplementation of ubiquinone, indicating that at least one of the signals for Cpx during LCFA metabolism is redox-dependent (Jaswal et al. 2020).

The outer membrane lipoprotein, NlpE, is a well-recognized signal for Cpx activation (Delhaye et al. 2019; Snyder et al. 1995). Recent observations that NlpE is a DsbA substrate, disruption of its C-terminal domain disulfide bond activates Cpx, and Cpx induction in Δ*dsbA* is NlpE-dependent, have suggested NlpE to be a sensor of oxidative protein folding defects (Delhaye et al. 2019). However, NlpE is not the molecular cue for Cpx during LCFA metabolism; Cpx is fully induced in Δ*nlpE* grown in oleate (Jaswal et al. 2020). A possible redox signal during LCFA metabolism is the periplasmic chaperone-protease DegP. The function of DegP is redox-dependent; its disulfide-bonded form is a chaperone, whereas the thiol form is a protease (Skorko-Glonek et al. 2008). One of the substrates of DegP protease is CpxP, a negative regulator of the Cpx response (Buelow and Raivio 2005; Isaac et al. 2005). Since DegP accumulates significantly in its thiol form in LCFA-utilizing cells (Jaswal et al. 2020), the reduced form of DegP may degrade CpxP to activate Cpx. Further, the other known inducers of Cpx, i.e., the inner-membrane respiratory complexes and lipoproteins other than NlpE (Guest et al. 2017; Miyadai et al. 2004), may constitute the redox-dependent or redox-independent signals for Cpx activation during growth in LCFAs. Clearly, detailed studies are needed to identify the Cpx-inducing signals in LCFA-utilizing cells and investigate their interplay that leads to robust Cpx activation.

Several mechanisms can be envisaged for the maintenance of envelope redox homeostasis by Cpx during LCFA metabolism: (i) Cpx response decreases envelope stress by repairing/degrading damaged proteins that accumulate due to inadequate disulfide bond formation, (ii) Cpx reduces the load on ETC by decreasing electron flow from LCFA metabolism, thereby increasing the availability of ubiquinone for disulfide bond formation, and (iii) Cpx facilitates electron transfer from disulfide bond-forming machinery by increasing the oxidizing power of ETC (Fig. 3). The following existing information on the Cpx pathway lends support to these suggested mechanisms: (i) Cpx upregulates several periplasmic chaperones, proteases and their modulators, and peptidyl-prolyl isomerases (Raivio et al. 2013), (ii) Cpx downregulates NADH dehydrogenase I and succinate dehydrogenase (Guest et al. 2017; Raivio et al. 2013).

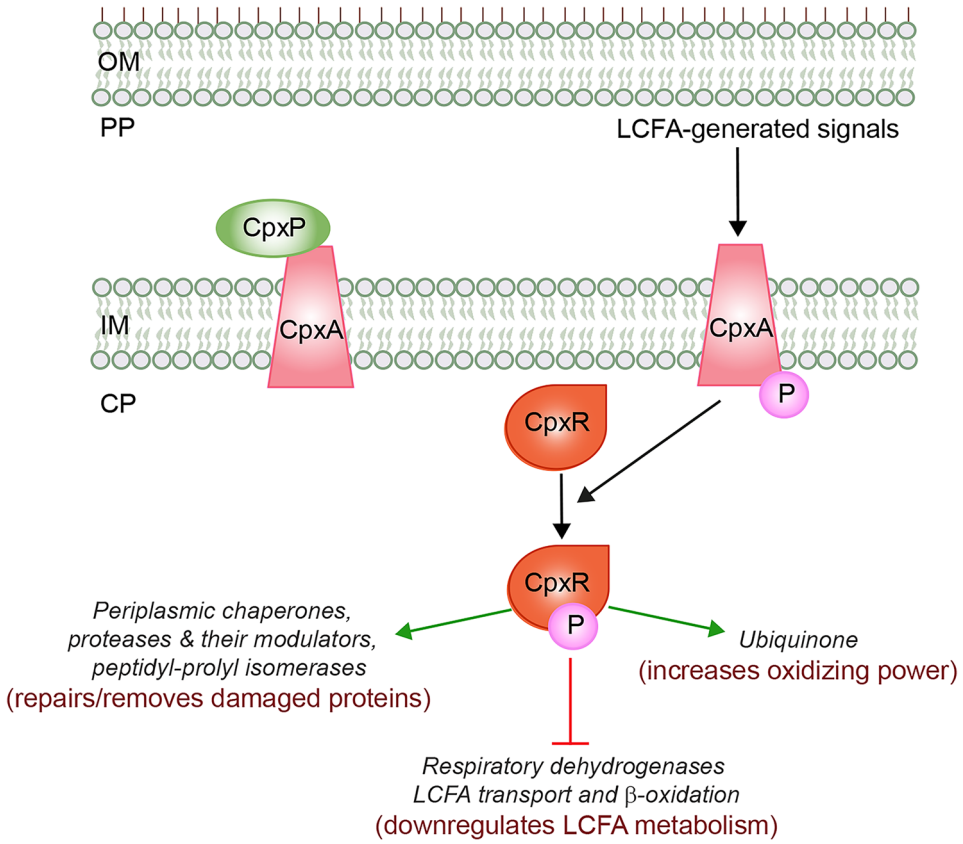

Fig. 3 Probable mechanisms by which Cpx restores disulfide bond formation in LCFA-grown cells. The CpxAR two-component system is comprised of an inner membrane histidine kinase, CpxA, and a cytoplasmic transcriptional regulator, CpxR. Under unstressed conditions, a periplasmic protein, CpxP, inhibits the phosphorylation of CpxA. In the presence of LCFA-generated stress signals, CpxA autophosphorylates and transfers its phosphoryl group to CpxR. The activation of the Cpx pathway decreases envelope stress by upregulating periplasmic chaperones, proteases and their modulators, and peptidyl-prolyl isomerases, which repair/remove damaged proteins. Besides, Cpx increases the oxidizing power for disulfide bond formation by upregulating ubiquinone and further increases ubiquinone availability for oxidative protein folding by downregulating the components of LCFA metabolism. *CP* cytoplasm, *IM* inner membrane, *PP* periplasm, *OM* outer membrane

Further, in the Δ*cydD* strain, which has a hyper-oxidizing envelope (a situation inverse of LCFA metabolism), Cpx is downregulated, whereas *fad* genes are upregulated (Goldman et al. 1996; Holyoake et al. 2016; Jaswal et al. 2020; Messens et al. 2007), and (iii) the upregulation of ubiquinone in LCFA-utilizing cells is abrogated in a Δ*cpxR* strain (Jaswal et al. 2020).

Detailed studies are available on the regulation of LCFA metabolism by FadR and the ArcA–ArcB two-component system. Both FadR and the cytoplasmic response regulator ArcA repress *fad* genes, and ArcA additionally regulates ETC components (Bongaerts et al. 1995; Cho et al. 2006; Cotter and Gunsalus 1992; Feng and Cronan 2012; Fujita et al. 2007; Iuchi and Lin 1988; Kwon et al. 2005; Zhang and Javor 2003). Importantly, the activity of the inner-membrane sensor kinase ArcB is governed by the redox state of quinones (Alvarez et al. 2013; Georgellis et al. 2001). Since Cpx upregulates ubiquinone in LCFA-utilizing cells (Jaswal et al. 2020), it is possible that the effect of ArcA–ArcB on LCFA metabolism is modulated by Cpx. Understanding the feedback exerted by Cpx in LCFA-grown cells and its crosstalk with FadR and ArcA–ArcB under these metabolic conditions is an exciting area for future research.

Concluding remarks

Recent work on the interconnection between LCFA metabolism and stress responses in *E. coli* opens up several new areas of investigation. In the immediate future, studies will be required to identify the major site of ROS formation, understand the mechanism by which ubiquinone counteracts LCFA-induced oxidative stress, and identify the molecular signal for Cpx activation and investigate the nature of its feedback. Further, it will be important to examine whether bacterial pathogens use mechanisms equivalent to those

identified in *E. coli* to defend against the harmful effects of LCFAs.

A pertinent question is why despite being predisposed to LCFA-induced stresses, bacteria readily use this nutrient source during infection. Does the use of LCFAs precondition bacteria to external stresses encountered in the host? In fact, a recent study demonstrated that hypoxia is less stressful for *M. tuberculosis* cultured in LCFAs, suggesting that inside the foamy macrophages, LCFA utilization enables the tubercle bacilli to survive environmental stresses (Del Portillo et al. 2018). During infection, bacteria often encounter ROS from the host immune system, such as the oxidative burst associated with neutrophils and phagocytes (Papp-Szabo et al. 1994; Rhen 2019). LCFA-induced ROS formation and the accumulation of ubiquinone might be a priming mechanism by which bacteria adapt to oxidative stress from the host. Notably, ubiquinone biosynthesis mutants of *S.* Typhimurium are impaired for intracellular proliferation in macrophages (Aussel et al. 2014a; Loiseau et al. 2017). Since during infection, *S.* Typhimurium uses LCFAs in macrophages (Fang et al. 2005; Mahan et al. 1995), it is plausible that LCFA-induced upregulation of ubiquinone serves to combat ROS produced by these immune cells.

The Cpx pathway governs many cellular processes in Gram-negative bacteria. Cpx affects biofilm formation, motility, and chemotaxis in *E. coli* and *Salmonella* Enteritidis by modulating the expression of structural, biogenesis, and regulatory components of flagella and curli (De Wulf et al. 1999; Dorel et al. 1999; Prigent-Combaret et al. 2001; Raivio 2014; Raivio et al. 2013; Shetty et al. 2019). Further, Cpx regulates the expression and assembly of cell-surface structures associated with virulence, such as pili of uropathogenic and enteropathogenic *E. coli* strains (Herndday et al. 2004; Hung et al. 2001; Nevesinjac and Raivio 2005), and controls the invasiveness of *S.* Typhimurium and *Shigella sonnei* in a pH-dependent manner by regulating the expression of type III secretion system (Humphreys et al. 2004; Nakayama and Watanabe 1995; Nakayama et al. 2003). The Cpx response is also associated with antibiotic resistance [reviewed in Raivio (2014)]. It is possible that LCFA-related problems in disulfide bond formation serve as a cue to induce Cpx to regulate cellular processes, which ultimately affect bacterial survival in the host.

Future research in the aforementioned directions will provide insights on how LCFA metabolism is integrated with stress responses and enable a deeper understanding of its impact on host-bacterial interactions.

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