Title:	A proteomics approach to identify the molecular signals for cpx activation during long-chain fatty acid metabolism in escherichia coli
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Abstract:	Gram-negative bacteria like Escherichia coli are able to thrive in a multitude of environments owing to the presence of a cell-envelope that protects them from various environmental insults. Since the envelope acts as the first line of defense and is crucial to cell survival, its integrity is continuously monitored and maintained by envelope stress response (ESR) pathways. The periplasm, an aqueous component of the bacterial cell-envelope between the inner and outer-membranes, bears a hyper-oxidizing environment that hosts many enzymes for a myriad of vital physiological processes. Amongst the wide range of diverse cellular processes that occur in the periplasm, it facilitates oxidative protein folding in several extracytoplasmic proteins. Previous work from our lab has shown that metabolism of long-chain fatty acids (LCFAs), a rich nutrient source for many bacteria including E.coli, shifts the redox environment of the periplasm towards a hypo-oxidising state, thereby hampering disulfide bond formation. Interestingly, an ESR pathway, CpxAR two-component system, gets activated in response to such LCFA-induced envelope stress. However, the molecular signals and mechanistic details for Cpx-activation during LCFA metabolism are still unknown, although a bimodal nature of activation is suggested that may be both redox- dependent and independent. This study focuses on uncovering the players involved in Cpx activation using a proteomics approach. The important players of the Cpx pathway, CpxA and CpxP, were SPA (Sequential Peptide Affinity) tagged to find their interacting partners during LCFA metabolism. Chromosomally tagged CpxA was observed in sufficient amounts that could be used for protein pulldown, however, tagged CpxP had to be cloned onto an inducible plasmid to be produced in detectable amounts. Protein profiles obtained from tagged strains grown with and without LCFAs were very similar and therefore advanced techniques like mass spectroscopy will be required to identify the specific players. The study also briefly discusses the standardization of conjugation, which can be used to screen ordered gene libraries, another strategy by which genes involved in the activation of the Cpx pathway can be uncovered.
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