Title:	Construction of Chromosomallly tagged strains of genes involved in ubiquinone (ubi) biosynthesis to assess Ubi protein levels during long-chain fatty acid metabolism in Escherichia coli
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Abstract:	Metabolic energy powers all living organisms, including gram negative bacteria like Escherichia coli. During carbon metabolism, reduced cofactors are generated, which get oxidized in the electron transport chain (ETC), and adenosine triphosphate (ATP) is produced by oxidative phosphorylation. The metabolism of long-chain fatty acids (LCFAs), a rich source of nutrients for many bacteria, including E. coli, is the focus of our lab's research. LCFAs are carboxylic acids with long, unbranched aliphatic chains of 12–20 carbon atoms. Our lab work has previously shown that LCFA metabolism generates a copious number of reduced cofactors, whose oxidation increases electron flow in the ETC. Ubiquinone, a lipid-soluble electron carrier in the ETC, rapidly transfers these electrons from respiratory dehydrogenases to terminal oxidases for further generation of ATP. Gram-negative bacteria, such as E. coli, can flourish in a wide range of environmental conditions due to the presence of a cell-envelope that protects them from a variety of external shocks. The bacterial cell envelope is constantly under check and is monitored by dedicated pathways called envelope stress response pathways (ESR). Besides taking up electrons from carbon metabolism, ubiquinone plays a pivotal role in the uptake of electrons from the disulfide bond (DSB) forming machinery, which facilitates the formation of DSBs in many periplasmic proteins in the oxidizing environment of the bacterial cell envelope. Work from our lab has revealed that the production of a large number of electrons during LCFA metabolism makes ubiquinone limiting for its electron transfer function and confers redox stress in E. coli. Importantly, the LCFA-induced redox-imbalance in the envelope is sensed by the Cpx ESR pathway which restores envelope redox homeostasis. Work from our lab suggests that an effective mechanism by which Cpx maintains homeostasis during LCFA metabolism is by increasing the oxidizing power of the cell by upregulating ubiquinone. Interestingly, E. coli grown in the presence of LCFA upregulates ubiquinone levels ~2 fold. However, the mechanistic details underlying this regulation remain elusive. The present study focuses on investigating the regulation on the genes involved in ubiquinone biosynthesis (ubi genes) pathway. For this, chromosomally tagged constructs for various ubi genes were created to monitor their protein levels in the presence of LCFAs. To ensure that tagging does not affect bacterial physiology, growth profiles of each tagged strain were compared to the untagged E coli BW25113 strain. All the tagged constructs had a growth profile similar to that of the WT. These constructs will be further used to assess protein levels of various Ubi proteins via Western Blotting.
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