Title:	Chromosomal tagging of genes involved in ubiquinone biosynthesis in Escherichia coli with Sequential Peptide Affinity (SPA) tag
Authors:	Malik, Shaheen
Keywords:	Chromosomal biosynthesis Sequential Peptide
Issue Date:	May-2024
Publisher:	IISER Mohali
Abstract:	All organisms, including bacteria, require energy to perform various cellular functions. The energy inside the cell is derived from metabolic processes that break down the nutrients acquired by the cell to yield growth intermediates and reduced cofactors (NADH and FADH 2). Respiratory dehydrogenases oxidize these reduced co-factors in the electron transport chain (ETC), which itself gets oxidized by transferring electrons to ubiquinone, a lipid-soluble electron carrier in the ETC. Ubiquinol, the reduced ubiquinone, further transfers electrons to terminal oxidases. This electron transfer through ETC builds up a proton gradient across the cellular membrane, leading to adenosine triphosphate (ATP) production via oxidative phosphorylation. Bacteria can utilize several carbon sources for ATP generation. Our lab works on the metabolism of long-chain fatty acids (LCFAs), which represent a rich source of nutrients for several bacteria, including Escherichia coli. Published work from our lab in E. coli has shown that the generation of a large number of reduced cofactors during LCFA metabolism renders ubiquinone limiting to handle the increased flow of electrons in the ETC, causing redox stress. Notably, ubiquinone accumulates ~2-fold in LCFA-grown cells in the stationary phase, which aids in the restoration of redox equilibrium. Importantly, our work showed that the upregulation of ubiquinone during LCFA metabolism is dependent on an envelope stress response pathway, the CpxAR two-component system. However, the mechanistic aspects of this regulation remain unknown. Previously, amongst 15 genes involved in ubiquinone biosynthesis (ubi genes), a former student from our lab chromosomally tagged 5 ubi genes. In the present work, I have chromosomally tagged 8 ubi genes by sequential peptide affinity (SPA) tag. These tagged constructs further can be exploited to understand the regulation of the Cpx pathway on ubi genes by determining their protein levels via Western blotting.
URI:	http://hdl.handle.net/123456789/5790
Appears in Collections:	MS-19

Files in This Item:

File	Description	Size	Format	
embargo period.pdf		6.04 kB	Adobe PDF	View/Open

Show full item record