Abstract

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Since decades riboflavin synthetic derivatives were used for probing the biological importance of different structural motifs of the molecule. Riboflavin analogs lacking one methyl group (7α or 8α) can still serve as a surrogate for riboflavin in riboflavin-deficient microorganisms or animals. The absence of both methyl groups at once completely abolishes this substitution capability. To elucidate the molecular mechanisms behind this phenomenon, we performed an adaptive laboratory evolution experiment (in triplicate) on an *E. coli* strain auxotrophic for riboflavin. As a result, the riboflavin requirement of the *E. coli* strain was reduced ~10-fold in the presence of 7,8-didemethyl-riboflavin. The whole genome sequencing of *E. coli* strains isolated from three experiments revealed two mutation hotspots: *lpd*A coding for the flavoenzyme dihydrolipoyl dehydrogenase (LpdA), and *omp*F coding for the major outer membrane protein. In order to investigate the essentiality of flavin's methyl groups to LpdA, the wild type and mutant variants of *lpd*A were cloned. At least two *lpd*A mutants increased the fitness of *E. coli*, and when 7,8-didemethyl-flavin was added to the growth medium, the increase was significant. To the best of our knowledge, an adaptive laboratory evolution experiment running in triplicate as a tool for the identification of mutation hotspots in the genome of microorganisms exposed to metabolic stress challenges is described here for the first time.