Engineering glyceraldehyde-3-phosphate dehydrogenase for sensitive regulation of glycolysis in *E. coli*

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Glycolysis is the most significant metabolic pathway for growth and maintenance of living organisms. Though it is designed highly robust by nature, relevant modification of glycolysis is important in metabolic engineering for the product accumulation, and it is considered as one of main technical challenges for the strain improvement. *gapA* is a one of the key glycolytic genes and essential for growth of cells on glucose. *gapA*-encoded protein, glyceraldehyde 3-phosphate dehydrogenase (GAPDH), is a tetrameric enzyme which mediates reversible oxidative phosphorylation of glyceraldehyde 3-phosphate into 1,3-diphosphoglycerate and has vital role in both glycolysis and gluconeogenesis. Wild-type GAPDH has strong activity and it is not easy to control its expression level by the conventional controllable promoters. In this study, we developed gapA mutants for the sensitive regulation of glycolysis in *E. coli* by directed evolution approach using gapA-deleted mutant as the base strain and temperature sensitive promoter system as the protein expression system. Several mutants with the different levels of regulation sensitivities were obtained, and relationship of activity and sequence was examined.