Genome-scale chromosomal engineering of bacterial hosts for bioenergy production

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Rational engineering of bacteria for optimal biofuel production requires understanding the relationships between genome arrangement, chromosome architecture, and gene expression. For instance, bacterial conversion of lignocellulosic feedstocks to biofuels must balance two disparate processes whose distinct gene expression and energetic requirements are intertwined with chromosome architecture: (1) the energetically costly synthesis and secretion of enzymes like cellulases that are required to release sugars from lignocellulose, which is best accomplished aerobically where energy for protein synthesis is most readily available; and (2) the metabolic conversion of the sugars to biofuels with maximal conservation of reducing equivalents, which is best accomplished anaerobically. Although poorly understood, the switch from aerobic to anaerobic growth is coupled to changes in bacterial chromosomal architecture. The nucleoid protein HU, which interacts with transcriptionally active DNA loops anchored at their base by a second nucleoid protein H-NS, promotes aerobic expression of genes that are repressed upon switch to anaerobic growth. We are studying the relationships between chromosome architecture, aerobic gene expression, and anaerobic gene expression in *E. coli*. Genome-scale analyses of gene-expression patterns, nucleoid-protein and transcription-regulator distributions, and cellular protein composition are being combined to elucidate these relationships. A full understanding will allow rational engineering of aerobic and anaerobic gene expression for optimal biofuel production by rational engineering of chromosomal gene location and transcriptional regulation.