Systematic discovery of uncharacterized transcription factors in Escherichia coli K-12 MG1655

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Transcriptional regulation enables cells to respond to environmental changes. Of the estimated 304 candidate transcription factors (TFs) in Escherichia coli K-12 MG1655, 185 have been experimentally identified, but ChIP methods have been used to fully characterize only a few dozen. Identifying these remaining TFs is key to improving our knowledge of the E. coli transcriptional regulatory network (TRN). Here, we developed an integrated workflow for the computational prediction and comprehensive experimental validation of TFs using a suite of genome-wide experiments. We applied this workflow to (i) identify 16 candidate TFs from over a hundred uncharacterized genes; (ii) capture a total of 255 DNA binding peaks for ten candidate TFs resulting in six high-confidence binding motifs; (iii) reconstruct the regulons of these ten TFs by determining gene expression changes upon deletion of each TF and (iv) identify the regulatory roles of three TFs (YiaJ, YdcI, and YeIE) as regulators of L-ascorbate utilization, proton transfer and acetate metabolism, and iron homeostasis under iron-limited conditions, respectively. Together, these results demonstrate how this workflow can be used to discover, characterize, and elucidate regulatory functions of uncharacterized TFs in parallel.

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