A systems approach discovers the role and characteristics of seven LysR type transcription factors in Escherichia coli

Irina A. Rodionova1,2,6*, Ye Gao1,2,6, Jonathan Monk1, Ying Hefner1, Nicholas Wong2, Richard Szubin1, Hyun Gyu Lim1, Dmitry A. Rodionov3, Zhongge Zhang2, Milton H. Saier Jr.2 & Bernhard O. Palsson1,4,5*

Although Escherichia coli K-12 strains represent perhaps the best known model bacteria, we do not know the identity or functions of all of their transcription factors (TFs). It is now possible to systematically discover the physiological function of TFs in E. coli BW25113 using a set of synergistic methods; including ChIP-exo, growth phenotyping, conserved gene clustering, and transcriptome analysis. Among 47 LysR-type TFs (L TFs) found on the E. coli K-12 genome, many regulate nitrogen source utilization or amino acid metabolism. However, 19 L TFs remain unknown. In this study, we elucidated the regulation of seven of these 19 L TFs: YbdO, YbeF, YcaN, YbhD, YgfI, YiaU, YneJ. We show that: (1) YbdO (tentatively re-named CitR) regulation has an effect on bacterial growth at low pH with citrate supplementation. CitR is a repressor of the ybdNM operon and is implicated in the regulation of citrate lyase genes (citCDEFG); (2) YgfI (tentatively re-named DhfA) activates the dhaKLM operon that encodes the phosphotransferase system, DhfA is involved in formate, glycerol and dihydroxyacetone utilization; (3) YiaU (tentatively re-named LpsR) regulates the yiaT gene encoding an outer membrane protein, and waaPSBOJYZU operon is also important in determining cell density at the stationary phase and resistance to oxacillin microaerobically; (4) YneJ, re-named here as PtrR, directly regulates the expression of the succinate-semialdehyde dehydrogenase, Sad (also known as YneI), and is a predicted regulator of fnrS (a small RNA molecule). PtrR is important for bacterial growth in the presence of l-glutamate and putrescine as nitrogen/energy sources; and (5) YbhD and YcaN regulate adjacent y-genes on the genome. We have thus established the functions for four L TFs and identified the target genes for three L TFs.

Recently, the roles of uncharacterized LysR-type transcription factors (LTFs) have been identified via multiple approaches, including transcriptome analysis of uncharacterized TF (yTF)-deleted mutants (machine-learning-based), gene clustering, and the detection of DNA-binding sites1. The predicted yTF targets annotated as transporters and enzymes define the TF physiological role. A combination of the knowledge from EcoCyc, Fitness Browser, and iModulonDB with TF DNA-binding data provided hypotheses for the putative physiological functions of yTFs under different growth conditions2. Indeed, as an example, the function of one TF, PunR, was discovered to be an activator for punC, purine transporter important for adenosine utilization as a nitrogen source. Recently novel regulators for csgD were found by the promoter-specific screening of the 198 purified TFs including yTFs: YbiH, YdcI, YbjC, YiaU, YgiL and YjiR. Two regulators for csgD: repressor YiaJ and activator YbjC (renamed RcdB) were found3. The SELEX method for the detection of the YbiH DNA-binding sites and antibiotics sensitivity for the yTF deletion mutant were used to predict the function for the CecR(YbiH)4.

The RNA-seq data for the TF deletion mutants under specific growth conditions provide information about transcription affected by mutation (Fig. 1). In Escherichia coli, LTFs are regulators for amino acids (AAs), purine,
and dicarboxylate metabolism, nitrogen assimilation (NAC), antibiotic resistance, and virulence. LTF regulatory proteins protect 50–60 bp regions with TA-rich regulatory binding sites and activation binding sites (ABS) and regulate different metabolic pathways. LTFs are known to regulate conserved gene clusters that are adjacent to the genes encoding the regulator, but additionally, LTF autoregulation is a common property. As one of the largest families of HTH-type regulators, LTFs contain an N-terminal helix-turn-helix DNA-binding domain and C-terminal co-inducer binding domain (Fig. S1). Given the broad conservation of LTFs, it is possible that they regulate a wide variety of target genes with diverse physiological functions using common regulatory features.

LTFs account for 16.7% (47 out of 280) of the total number of transcription factors in Escherichia coli K-12. Out of the 47 LTFs, 26 (AbgR, AllS, ArgP, Cbl, CynR, CysB, Dan, DmlR, DsdC, GcvA, HcaR, HdfR, IlvY, LeuO, LrhA, LysR, MetR, Nac, NhaR, OxyR, PerR, PgrR, QseA, QseD, TdcA, XapR) have known functions and the majority were shown to regulate adjacent genes. However, there are still many uncharacterized LTFs belonging to the LysR-type family, which require further studies to determine their regulatory functions. This effort is also important for the reconstruction of the transcriptional regulatory network (TRN). The set of six yTFs from LysR family (YbdO, YgfI, YcaN, YbhD, YiaU) upregulated in the presence of l-threonine as supplement (iModulonDB, PRECISE) to minimal growth medium and YneJ, transcriptional analysis for the regulation of phosphoenol-l-glutamate utilization in minimal medium were characterized in our research.

Escherichia coli is a representative of the commensal mammalian intestinal microbiota and is the best characterized model gram-negative bacterium. Nutrient starvation conditions are important for the gut microbiome bacterial community as they cause stress, activating different survival mechanisms, and TRNs rewire the metabolism under different conditions. iModulonDB is a collection of E. coli MG1655 transcriptomics data for different growth medium and stress conditions. Machine-learning based ICA data analysis (iModulonDB), RNA-seq data for the TF knockout, and ChIP-exo data (in vivo DNA-binding sites) are useful resources for LTF characterization. Recently, the ChIP-exo results for verified uncharacterized TFs in E. coli MG1655 was published. The gene expression profiling for LTFs under multiple growth conditions in iModulonDB provides important information for predicting the growth conditions to verification of function for the yTFs.

We performed systems analysis for the prediction of LTF functional role using data combined from previously published chromatin immunoprecipitation with exonuclease treatment (ChIP-exo) detected LTF DNA-binding sites, transcriptome data from iModulonDB (Fig. 1), RNA-seq analysis for LTF deletion mutants (experimental data), and genome cluster analysis using the bioinformatics tools (Fig. 1). We generated RNA-seq data for seven LTF mutants (ybdO, ygfI, ycaN, ybhD, yiaU, yneJ, and yneF) analyzed the conserved genome clustering with LTF genes, and detected conserved genes that were differentially expressed in the LTF knockout. Accordingly, we generated a hypothesis about the possible regulatory targets of three LTFs and their physiological functions.

For the detailed analysis, we investigated the effect of the yneJ transcriptional response in minimal medium under nitrogen starvation conditions. The yneJ-sad cluster is conserved in enterobacterial genomes, and Sad is involved in putrescine utilization as nitrogen source. Many bacterial species, including E. coli, can simultaneously utilize l-glutamate (Glu) and the polyamine putrescine (Ptr) under carbon/nitrogen starvation conditions.

Figure 1. Systems approach for the prediction of transcription factor’s function. A systematic approach to identify LysR family unknown transcription factors physiological function in E. coli.
Glu is also essential for tetrahydrofolate polyglutamylation. \(\text{Ptr}\) is important for bacterial growth and for efficient DNA replication, transcription, and translation and plays an important role in maintaining compact conformations of negatively charged nucleic acids. \(\text{Ptr}\) is also involved in multiple antibiotic resistance mechanisms under stress conditions. The \(\text{puu}\) genes, including \(\text{puu}\), are induced by \(\text{Ptr}\) and regulated at the transcriptional level by the \(\text{Ptr}\)-responsive repressor \(\text{Hnr}\). The expression of \(\text{sad}\) (\(\text{yeN}\)) is induced by the addition of \(\text{Ptr}\) to the medium; however, it is not regulated by \(\text{Puu}\) and is a transcriptional regulator for \(\text{sad}\) that had not been described before this work.

The predicted \(\text{Ptr}\) binding site in the \(\text{sad}\) promoter region was confirmed via an in vitro binding assay with the purified \(\text{Ptr}\) protein and using the ChIP-exo assay. We further compared the whole genome transcriptional response of the \(\text{ptr}\) knockout and wild type \(\text{E. coli}\) strains to carbon/nitrogen starvation in the presence of \(\text{Ptr}\) and l-glutamate using RNA-Seq analyses, and the \(\text{Ptr}\) DNA-binding site was predicted for \(\text{fnr}\), encoding small regulatory RNA. The physiological roles of \(\text{Ptr}\) regulation and antibiotic resistance are discussed.

**Results**

An integrated systems-approach uncovers LysR-type transcription factors in \(\text{E. coli}\) K-12. Previously, we generated a list of candidate transcription factors (TFs) from uncharacterized genes (\"y-genes\") using a homology-based algorithm. Among these TFs, it was predicted that \(\text{YE}\), \(\text{YER}\), \(\text{YID}\), \(\text{YAH}\), \(\text{YBD}\), \(\text{YBE}\), \(\text{YCA}\), \(\text{YCL}\), \(\text{YDH}\), \(\text{YEE}\), \(\text{YF}\), \(\text{YGI}\), \(\text{YHA}\), \(\text{YHJ}\), \(\text{YIA}\), \(\text{YNE}\), and \(\text{YNL}\) belong to the LT family on Hidden Markov Model. \(\text{YDH}\) (renamed \(\text{Punc}\)) function had been shown to be an activator for the purine transporter, \(\text{Punc}\). Recently \(\text{YDC}\) (\(\text{Salmonella enterica}\)) function was shown to be related to biofilm formation. We further chose 7 uncharacterized LysR-type TFs (\(\text{yTFs}\)), which have transcriptional responses (increased mRNAs) to the presence of a l-threonine (Thr) in M9 medium (iModulonDB, PRECISE). l-Threonine is an important source for l-serine, l-glycine, branched-chain amino acid biosynthesis, and formate, which is important for anaerobic respiration. To elucidate roles of each y-LTF and genome-wide target genes, we performed gene expression profiling via RNA-Seq and ChIP-exo detection of the LT DNA-binding and growth phenotyping. The overall workflow is shown in Fig. 1.

**YbdO (CitR) regulatory effects are involved in citrate utilization and YbeF is involved in the flagella biosynthesis.** The physiological function for LTfs CitR and YbeF was previously unknown. The YbdO (CitR) ChIP-exo result detected the peak for DNA-binding upstream of \(\text{citR}-\text{ybdNM}\) (Fig. 2A,B), that further was confirmed by the transcriptional analysis. The other ChIP-exo detected peaks for possible suggested genes regulation were not found in DEGs for \(\text{citR}\) deletion mutant strain. The \(\text{ptr}\) regulated by HNS and ybdNM operons are conserved adjacent genes in Enterobacteriaceae (Fig. 2D) and the ybdNM promoter was predicted to be regulated by FNR (EcoCyc). Further, gene expression profiling showed that \(\text{cit}\) deletion leads to strong upregulation of the \(\text{ybdNM}\) operon and the adjacent genes \(\text{citCDEF}\). Interestingly that genome context analysis evolved that \(\text{cit}\) is conserved with the citrate lyase encoding genes cluster \(\text{citCDEF}\) (Fig. 2C). Therefore, this result confirmed autoregulation of \(\text{citR}-\text{ybdNM}\), and regulation for citrate lyase which is involved in anaerobic metabolism. From PRECISE2, upregulation of \(\text{citC}\) was detected when \(\text{E. coli}\) MG1655 strain was grown in M9 medium supplemented with Thr, suggesting that citrate utilization is important for the catabolism of Thr. The YbdM has been predicted yTF (Uniprot) and probably related to flagellar transcription regulation and growth phenotyping. The overall workflow is shown in Fig. 1.

To test the regulation of the citrate lyase encoding gene cluster (\(\text{citEFG}\)), the phenotype for growth in the M9 medium supplemented with citrate at low pH (pH 6.5) showed an effect of \(\text{cit}\) deletion on growth, suggesting artificial de-repression of citrate lyase (Fig. 2E-F), but no difference for the growth in M9 medium supplemented with citrate at low pH (pH 6.5). However, \(\text{cit}\) mutation decreases the phenotype for motility in LB medium and affects growth using glycolate as carbon source (Fitness Browser, fitdb). Interestingly that genome context of the \(\text{cit}\) operon and the adjacent genes \(\text{citCDEF}\). \(\text{cit}\) deletion leads to downregulation on citrate utilization, flagellar transcription regulation and growth phenotyping.

**YcaN and Ybd regulators mutant characterization.** The \(\text{ybd}\) and \(\text{yca}\) mutant strains and WT were collected at the late-exponential phase after growth in the M9 medium supplemented with Thr. The conserved gene cluster \(\text{ycaN-ycaK-ycaM}\) with adjacent \(\text{yce}\) and \(\text{yca}\) genes was detected in the Escherichia coli K12 and Shigella boydii genomes. We noticed that in the \(\text{yca}\) deletion mutant strain differentially expressed genes \(\text{yca}\) (downregulated) and \(\text{yca}\), \(\text{yce}\) (encoding formate channel) (upregulated) are genes adjacent to \(\text{yca}\). It is interesting that \(\text{yca}\) and \(\text{yce}\) are predicted to be regulated by Nac (belonging to LT), as Nac function is nitrogen assimilation, and \(\text{yca}\) and \(\text{yce}\) are probably nitrogen assimilation function related (EcoCyc). In \(\text{yca}\) deletion mutant highly DEGs are the \(\text{maa}\) genes, encoding \(\text{i}\)-tryptophanase and a tryptophan transporter, \(\text{i}\)-arginine degradation, \(\text{astCADBE}\), an autotransporter-2 transport system (\(\text{isrABC}\)), and the \(\text{HIT}\)-type transcriptional regulator, \(\text{galS}\), the genes were strongly downregulated (Supplementary Data 1). The genes encod-
ing amino acid metabolism (l-valine biosynthesis (IlvB, IlvN), threonine dehydrogenase (Tdh), transcriptional activator (TdcA), and glycolate utilization pathway (GlcC, GlcD, GlcF)) were additionally downregulated in the ycaN mutant. The ycaN mutant phenotype microarray applied for 95 carbon sources had evolved the negative phenotype for formate utilization specifically suggesting YcaN dependent fecA regulation involved formate utilization (Fig. 7).

The ybbH gene is divergently oriented with respect to the conserved Enterobacterial gene cluster ybbHI and the putative hydrolase gene, ybbJ (Fig. S5A). YbbH is a 4-oxalomesaconate tautomerase homologous protein, and YbbH is a putative tricarboxylate transporter, homologous to 2-oxoglutarate/malate translocator, (id. 35%) (iModulonDB) (Fig. S5B). The YbhH encoding gene was strongly upregulated in a ybbD deletion mutant compared to the wild type DW25113 strain of E. coli in 96-well plates microaerobically in M9 glucose supplemented by 30 mM citrate at pH 7.5 (orange and grey line) or glycerol as carbon source (blue and yellow line). (F) Growth was measured in 96-well plates (M9 with glycerol) as the carbon source, supplemented by 30 mM citrate at pH 6.5 (WT-orange and ybbD mutant strain-blue line).

YgfI (Dhfa) regulation and glycerol and formate utilization. ChIP-exo assays previously detected Dhfa binding upstream of the dhaKLM operon encoding the dihydroxyacetone phosphotransferase (DHAK) (Fig. 3A-B). The transcriptional activation of DhAKLM is important for glycerol utilization and M9 supplemented by Thr or L-tryptophan (Fig. 3B) (iModulonDB). DHAK in the E. coli MG1655 strain is involved in glycerol utilization (UniProt). Accordingly, we decided to test the effect of the dhfa deletion on the growth on glucose (Fig. 3C-D) and glycerol (Fig. 3E). The resulting deficiency in growth on glycerol is potentially explained by dhAKLM as well as pfB, hyBCD, hycEFG, and adhB transcriptional Dhfa activation, as shown by RNA-seq (Table 2), and the Dhfa binding site was predicted upstream of those genes (Table S1). The DEGs detected by RNA-seq showed substantial downregulation of formate fermentation related genes (Fig. 3F) encoding pyruvate-formate lyase (pfB), fumarate reductase (frdABCD), formate hydrogenlyase (hyBCD, hycEFG), and the regulator (hyCA), as well as hybABC, hybEF, and hybO and the gene encoding protein involved in maturation of all hydrogenases isoyzemes (hybP) (Fig. 3F) and the dhAKLM operon (Table 2, Supplementary Data). Dhfa deletion had little effect on growth using glucose as the carbon source (Fig. 3C) and with Thr supplement (Fig. 3D) aerobically, but the dhfa strain had a low growth rate microaerobically without Thr supplement in minimal medium (Fig. S7).

The detected Dhfa binding upstream of dhAKLM and the defect for the growth on glycerol (Fig. 3E) for the dhfa mutant confirm the Dhfa-dependent transcriptional activation of DHAK, as DHAK is involved in glycerol
| Gene name | Gene function | p value | Base Mean | log<sub>2</sub> Fold Change |
|-----------|---------------|---------|-----------|--------------------------|
| b2289     | hBA DNA-binding transcriptional dual regulator LrhA | 3.89E−26 | 145 | −4.3 |
| b1496     | yddA ABC transporter family protein YddA | 0.000511 | 13 | −3.2 |
| b4462     | ygaQ Putative uncharacterized protein YgaQ | 4.66E−05 | 15 | −3.0 |
| b2368     | emrK Tripartite efflux pump membrane fusion protein EmrK | 0.000211 | 16 | −2.8 |
| b2273     | yfbN Uncharacterized protein YfbN | 0.000348 | 16 | −2.5 |
| b2845     | yqcG | 2.13E−05 | 24 | −2.5 |
| b2373     | oxc Oxalyl-CoA decarboxylase | 0.00095 | 11 | −2.1 |
| b34660_1  | yhiL | 0.001038 | 122 | −1.4 |
| b3043     | ygiL | 0.00100 | 10 | −1.6 |
| b2309     | hisJ Histidine ABC transporter periplasmic binding protein | 0.000348 | 216 | −1.4 |
| b3025     | hisP Lysine/arginine/ornithine ABC transporter/histidine ABC transporter, ATP binding subunit | 4.90E−05 | 264 | −1.4 |
| b1025     | dgcT | 2.37E−05 | 168 | 1.2 |
| b0854     | potF | 5.59E−07 | 585 | 1.2 |
| b2306     | argT | 0.000279 | 291 | 1.3 |
| b34661_1  | fliR Flagellar biosynthesis protein Flir | 5.00E−08 | 297 | 1.3 |
| b2751     | fliN Flagellar biosynthesis protein Flin | 1.16E−08 | 315 | 1.3 |
| b2764     | cysE Sulfate/thiosulfate ABC transporter inner membrane subunit CysE | 4.00E−05 | 381 | 1.8 |
| b2242     | cysU Sulfate/thiosulfate ABC transporter inner membrane subunit CysU | 1.29E−05 | 245 | 2.0 |
| b1950     | flkB Flagellar biosynthesis protein FlkB | 0.000674 | 20 | 2.3 |
| b2751     | cysN Sulfur adenylyltransferase subunit 1 | 2.77E−08 | 297 | 2.4 |
| b1879     | flhA Flagellar biosynthesis protein FlhA | 1.37E−09 | 190 | 2.5 |
| b2752     | cysD Sulfur adenylyltransferase subunit 2 | 2.03E−08 | 249 | 2.5 |
| b2467     | ybdM ParB-like nucleosome-containing protein YbdM | 0.000248 | 20 | 2.5 |
| b2764     | cysI Sulfite reductase flavoprotein subunit | 1.62E−08 | 381 | 2.6 |
| b2750     | cysC Adenylyl-sulfate kinase | 1.34E−08 | 162 | 2.6 |
| b4110     | yscZ Uncharacterized protein YscZ | 2.81E−08 | 351 | 2.7 |
| b2422     | cysA Sulfate/thiosulfate ABC transporter ATP binding subunit | 4.46E−08 | 296 | 2.7 |
| b2763     | cysL Sulfite reductase hemoprotein subunit | 3.02E−09 | 567 | 2.8 |
| b1070     | flgN Flagellar biosynthesis protein FlgN | 2.32E−06 | 338 | 2.9 |
| b1880     | flhB Flagellar biosynthesis protein FlhB | 4.53E−10 | 118 | 3.0 |
| b1071     | flgM Anti-sigma factor for FlgM | 7.52E−05 | 86 | 3.0 |
| b1946     | flsN Flagellar motor switch protein FlsN | 1.42E−05 | 30 | 3.1 |
| b1566     | flsA Flagellar biosynthesis protein FlsA | 8.91E−06 | 402 | 3.1 |
| b2762     | cysH Phosphoadenosine phosphosulfate reductase | 3.14E−09 | 145 | 3.2 |
| b3525     | pseH c-di-GMP phosphodiesterase PseH | 6.75E−11 | 176 | 3.3 |
| b1072     | flgA Flagellar basal body P-ring formation protein FlgA | 4.65E−13 | 225 | 3.3 |
| b0615     | citF Citrate lyase alpha subunit | 3.40E−08 | 20 | 3.3 |
| b1941     | fliB Flagellum-specific ATP synthase FliB | 1.66E−11 | 165 | 3.4 |
| b1948     | fliP Flagellar biosynthesis protein FlIP | 2.90E−10 | 40 | 3.5 |
| b1081     | fliQ Putative peptidoglycan hydrolase FliQ | 8.01E−15 | 126 | 3.5 |
| b1943     | fliK Flagellar hook-length control protein | 7.25E−10 | 87 | 3.7 |
| b1083     | fliL Flagellar hook-filament junction protein 2 | 5.67E−16 | 308 | 3.7 |
| b1080     | fliF Flagellar P-ring protein | 3.67E−17 | 263 | 3.7 |
| b1942     | fliI Flagellar biosynthesis protein FlII | 1.46E−05 | 27 | 3.7 |
| b1939     | fliG Flagellar motor switch protein FlIG | 3.18E−13 | 213 | 3.8 |
| b1940     | fliH Flagellar biosynthesis protein FlII | 1.09E−12 | 73 | 3.8 |
| b1938     | fliF Flagellar basal-body MS-ring and collar protein | 1.44E−24 | 645 | 3.8 |

Continued
tionally, ChIP-exo detected binding for the genes encoding adenine transporter, suggesting transcriptional activation in response to the stress. LpsR was found essential for the possible LTF (encoding lipopolysaccharide (LPS) biosynthesis glycero-d-manno-heptose kinase) (Fig. 4A). The results suggest that LpsR is important for LPS biosynthesis at specific conditions and the majority of DEGs encode the proteins involved in cell wall/membrane/envelope biogenesis, carbohydrate transport, energy production, and amino acid metabolism (Fig. 4B). Accordingly, gene expression for the lpsR mutant was lower for the genes from the operon waaPSBOJYZU, suggesting that LpsR is the LPS biosynthesis operon activator (Table 3); previously the regulator for waa operon was not known (ecocyc.org). Additionally, ChIP-exo detected binding for the genes encoding adenine transporter, adeP, suggests transcriptional regulation, and they were detected as DEGs for the yiaU mutant (Fig. 4D). We suggest re-naming YiaU to LpsR—LPS biosynthesis regulator. The Biolog plates with the antibiotics were tested microaerobically as was shown by the phenotype microarray analysis (Fig. 7).

YiaU (LpsR) regulatory network and yiaU mutant growth phenotype. ChIP-exo results show the LpsR binding for the regulation of waaP (encoding lipopolysaccharide (LPS) biosynthesis glycero-d-manno-heptose kinase) (Fig. 4A). The results suggest that LpsR is important for LPS biosynthesis at specific conditions and the majority of DEGs encode the proteins involved in cell wall/membrane/envelope biogenesis, carbohydrate transport, energy production, and amino acid metabolism (Fig. 4B). Accordingly, gene expression for the lpsR mutant was lower for the genes from the operon waaPSBOJYZU, suggesting that LpsR is the LPS biosynthesis operon activator (Table 3); previously the regulator for waa operon was not known (ecocyc.org). Additionally, ChIP-exo detected binding for the genes encoding adenine transporter, adeP, suggests transcriptional regulation, and they were detected as DEGs for the yiaU mutant (Fig. 4D). We suggest re-naming YiaU to LpsR—LPS biosynthesis regulator. The Biolog plates with the antibiotics were tested microaerobically as was shown by the phenotype microarray analysis (Fig. 7).

Table 1. Differentially expressed genes revealed by RNA-Seq ybdO deletion mutant strain and wild type E. coli strains during growth in M9 medium with glucose as the primary carbon source and 7 mM l-threonine as supplement.

| Gene name | Gene function | p value | Base Mean | log2 Fold Change |
|-----------|---------------|---------|-----------|-----------------|
| flIM      | Flagellar motor switch protein FliM | 3.39E−18 | 174       | 3.9             |
| flgG      | Flagellar basal-body rod protein FlgG | 2.24E−17 | 209       | 3.9             |
| cheY      | Chemotaxis protein CheY | 2.68E−11 | 232       | 4.0             |
| citC      | Citrate lyase synthetase | 3.64E−07 | 12        | 4.0             |
| fliA      | RNA polymerase sigma 28 (sigma F) factor | 8.52E−18 | 3038      | 4.0             |
| fliC      | Flagellar basal-body rod protein FlgC | 1.73E−19 | 130       | 4.1             |
| cheW      | Chemotaxis protein CheW | 1.74E−16 | 345       | 4.1             |
| fliL      | Flagellar protein FilL | 2.57E−11 | 46        | 4.1             |
| fliH      | Flagellar L-ring protein | 1.32E−16 | 115       | 4.2             |
| fliS      | Flagellar biosynthesis protein FliS | 4.36E−07 | 134       | 4.2             |
| tsr       | Methyl-accepting chemotaxis protein Tsr | 2.59E−21 | 2250      | 4.2             |
| fliE      | Flagellar filament structural protein | 3.74E−19 | 18,478    | 4.2             |
| flgB      | Flagellar basal-body rod protein FlgB | 2.75E−20 | 268       | 4.3             |
| flgF      | Flagellar basal-body rod protein FlgF | 2.31E−23 | 532       | 4.3             |
| cheZ      | Chemotaxis protein CheZ | 1.19E−26 | 381       | 4.3             |
| flgK      | Flagellar hook-filament junction protein 1 | 1.75E−21 | 1771      | 4.3             |
| fliD      | flagellar filament capping protein | 1.63E−16 | 623       | 4.3             |
| fisO      | Flagellar biosynthesis protein FisO | 7.05E−16 | 68        | 4.4             |
| citE      | Citrate lyase beta subunit | 7.69E−07 | 9         | 4.4             |
| cheR      | Chemotaxis protein methyltransferase | 1.17E−11 | 158       | 4.4             |
| flgD      | Flagellar biosynthesis initiation of hook assembly | 1.13E−21 | 400       | 4.5             |
| cheB      | Protein-glutamate methyltransferase/protein glutamine deamidase | 1.04E−20 | 415       | 4.5             |
| cheZ      | Chemotaxis protein CheZ | 1.74E−16 | 345       | 4.1             |
| flgE      | Flagellar hook protein FlgE | 1.14E−22 | 677       | 4.6             |
| motA      | Motility protein A | 4.60E−21 | 267       | 4.6             |
| tap       | Methyl-accepting chemotaxis protein Tap | 9.03E−23 | 1642      | 4.6             |
| cheA      | chemotaxis protein CheA | 1.33E−27 | 679       | 4.8             |
| motB      | Motility protein B | 5.67E−26 | 410       | 4.8             |
| tar       | Methyl-accepting chemotaxis protein Tar | 7.76E−31 | 1507      | 4.9             |
| flIT      | Flagellar biosynthesis protein FlIT | 0.000596 | 21        | 5.3             |
| ybdN      | Putative PAPS reductase/DUF3440 domain-containing protein | 1.86E−22 | 36        | 5.5             |

utilization22. We suggest re-naming YgfI to DhfA—dihydroxyacetone/formate utilization activator. The fermentation products during microaerobic growth on M9 glucose were detected in the Thr supplemented M9 medium. The analysis evolved the higher efflux of formate and acetate for the dhfA mutant strain and wild type E. coli strains during growth in M9 medium with glucose as the primary carbon source and 7 mM l-threonine as supplement.

https://doi.org/10.1038/s41598-022-11134-7
The LysR family representatives are known to regulate adjacent genes, and lpsR-yiaT are conserved in the bacterial genomes (Fig. 4E). The RNA-seq results for the lpsR deletion mutant strain showed upregulation of the yiaT gene, encoding a predicted outer membrane protein membrane anchor for the surface display for the proteins, homologue of MipA. MipA is an MltA (murein-degrading enzyme) interacting protein.

YneJ (PtrR) regulatory effects for sad and fnrS transcription and putrescine utilization. Two distinct Ptr utilization pathways are known for E. coli (Fig. 5A). The first is catalyzed by the PuuA, PuuB, PuuC, and PuuD enzymes encoded by the puuP, puuA, puuDR, puuCB, puuE gene cluster and involves degradation of Ptr to γ-aminobutyric acid (GABA) via γ-glutamylated intermediates. The alternative pathway of Ptr degradation to GABA consists of PatA (Ptr aminotransferase) and PatD (γ-aminobutyraldehyde dehydrogenase)24. The PuuABCDE pathway is essential for Ptr utilization in E. coli using PuuP as the major Ptr transporter25. GABA is further utilized by two alternative 4-aminobutyrate aminotransferases (GABA-AT) encoded by gabT and puuE, and also two succinate semialdehyde dehydrogenases (SSADH) encoded by gabD and sad26,27.

We decided to characterize in detail the YneJ (re-named PtrR) by analyzing thePtrR ChIP-exo detected binding sites1. The ptrR gene is located in a conserved gene cluster with the divergently transcribed sad (yneI) gene, which encodes succinate semialdehyde dehydrogenase and yneH (glsB) glutaminase (Fig. 6A and 6C), upregulated in the evolved yneJ mutant strain (iModulonDB)28. To identify and characterize DNA binding sites of PtrR in the E. coli genome we utilized a combined bioinformatics and experimental approach. First, we applied a comparative genomic approach of phylogenetic footprinting27 to predict putative PtrR-binding sites in the common intergenic region of the ptrR and sad genes (Fig. 6C, Fig. S8). The ptrR/sad genes are conserved in several taxonomic groups including Escherichia/Salmonella/Shigella, Citrobacter, Enterobacter, and Klebsiella, as well as in Pseudomonas spp. In E. coli and closely related enterobacteria the sad gene belongs to the putative sad-ynelH gene cluster, while in Enterobacter and Citrobacter the orthologous genes include an additional gene encoding the methyl-accepting chemotaxis protein 1 (serine chemoreceptor protein, Mcp) (Fig. 6C). The multiple sequence alignment of ptrR/sad upstream regions from E. coli and closely related enterobacteria (termed Group 1 species) contains a conserved 15-bp palindromic sequence with consensus TTCACnAATnGAGAA downstream.
| Gene name | Gene function | Base mean | log2 fold change |
|-----------|---------------|-----------|-----------------|
| b2724 hycB | Formate hydrogenlyase subunit HycB | 29.3 | −9.0 |
| b1557 capB | Qin prophage cold shock-like protein CapB | 48.8 | −8.3 |
| b1937 flfE | Flagellar basal-body protein FlfE | 66.8 | 7.7 |
| b0990 capG | cold shock protein CapG | 10.2 | −7.6 |
| b2727 hypB | Hydrogenase isoenzymes nickel incorporation protein HypB | 53.6 | −7.5 |
| b2921 yglF | Putative LysR-type transcriptional regulator | 21.6 | −7.3 |
| b1922 flaA | RNA polymerase sigma 28 (sigma F) factor | 3038.3 | 7.2 |
| b3556 capA | cold shock protein CapA | 247.8 | −7.2 |
| b0572 cystC | Copper/silver export system outer membrane channel | 1017.0 | 7.1 |
| b2720 hycF | Formate hydrogenlyase subunit HycF | 37.7 | −7.1 |
| b4335 yijM | Putative dehydratase subunit | 228.8 | −6.9 |
| b1904 yecR | Lipoprotein YecR | 6.0 | 6.6 |
| b1938 flaF | Flagellar basal-body MS-ring and collar protein | 645.3 | 6.5 |
| b1939 flaG | Flagellar motor switch protein FlgG | 213.0 | 6.5 |
| b2721 hycE | Formate hydrogenlyase subunit HycE | 154.6 | −6.6 |
| b1729 cysP | Cysteine/sulfocysteine:cation symporter | 654.8 | 6.3 |
| b4037 malM | Malate regulon periplasmic protein | 110.6 | −6.2 |
| b1409 ynbB | Putative CDF-diaphorase synthase | 2.4 | −6.1 |
| b1674 ydhY | Putative 4Fe-4S ferredoxin-type protein | 80.9 | −6.1 |
| b2728 hypC | Hydrogenase 3 maturation protein HypC | 5.7 | −6.1 |
| b1072 flgA | Flagellar basal body P-ring formation protein FlgA | 225.3 | 6.0 |
| b1886 tar | Methyl-accepting chemotaxis protein Tar | 1507.2 | 6.0 |
| b1258 ycfF | Uroporphyrinogen III cosynthase | 27.2 | 6.0 |
| b1566 flaA | Qin prophage protein FlA | 402.2 | 6.0 |
| b1887 cheW | Chemotaxis protein CheW | 345.4 | 6.0 |
| b2378 lpxP | Palmitoyl acyltransferase | 422.4 | −5.9 |
| b1073 flgB | Flagellar basal-body rod protein FlgB | 267.6 | 5.9 |
| b1375 ynaE | Rac prophage uncharacterized protein YnaE | 4.5 | −5.9 |
| b1923 flaC | Flagellar filament structural protein | 18,478.4 | 5.8 |
| b1880 flhB | Flagellar biosynthesis protein FlhB | 118.3 | 5.8 |
| b1942 flhC | Flagellar biosynthesis protein FlhC | 27.2 | 5.8 |
| b2971 ygbG | Lipoprotein YgbG | 3.4 | 5.8 |
| b4380 yifA | DUFS029 domain-containing protein YifA | 128.5 | −5.7 |
| b4034 malE | Malate ABC transporter periplasmic binding protein | 402.0 | −5.7 |
| b2997 hyoB | Hydrogenase 2 small subunit | 245.6 | −5.7 |
| b1241 adhE | Aldehyde-alcohol dehydrogenase | 8441.5 | 5.5 |
| b1925 flfS | Flagellar biosynthesis protein FlfS | 134.0 | 5.6 |
| b1587 ynfE | Putative selenate reductase YnfE | 250.8 | −5.6 |
| b1083 flgL | Flagellar hook-filament junction protein 2 | 3080.4 | 5.6 |
| b1552 capI | Qin prophage cold shock protein CapI | 6.3 | −5.6 |
| b3370 flaA | Fructose-6-phosphate-fructose-1,6-bisphosphatase | 2.1 | −5.5 |
| b1589 ynfG | Putative oxidoreductase YnfG | 87.0 | −5.5 |
| b1890 motA | Motility protein A | 267.0 | 5.4 |
| b1940 flhE | Flagellar biosynthesis protein FlhE | 73.1 | 5.4 |
| b0903 pflB | Pyruvate formate-lyase | 23,055.8 | −5.4 |
| b4355 ntr | Methyl-accepting chemotaxis protein Ntr | 2250.0 | 5.4 |
| b0849 gpxA | Reduced glutaredoxin 1 | 8.0 | 5.4 |
| b2995 hyoB | Hydrogenase 2 membrane subunit | 157.1 | −5.4 |
| b2723 hycC | Formate hydrogenlyase subunit HycC | 118.9 | −5.4 |
| b0894 dimA | Dimethyl sulfoxide reductase subunit A | 454.5 | −5.4 |
| b2722 hycD | Formate hydrogenlyase subunit HycD | 50.9 | −5.4 |
| b1944 flhL | Flagellar protein FlhL | 46.2 | 5.4 |
| b4334 yflL | Putative ATPase activator of (R)-hydroxyglutaryl-CoA dehydratase | 50.6 | −5.3 |
| b1946 flaN | Flagellar motor switch protein FlaN | 30.4 | 5.3 |
| b4036 lamB | Maltose outer membrane channel/phage lambda receptor protein | 191.8 | −5.3 |
| b1757 ynfE | Molybdoenzyme synthase sulfurtransferase | 355.9 | −5.3 |

Continued
predicted sigma-E dependent promoter (Fig. 6A). We also analyzed upstream regions of ptrR orthologs in other enterobacterial genomes (Group 2 species), where the sad gene ortholog is absent and ptrr is co-localized with an uncharacterized MFS-family transporter gene. Further, we predicted two conserved DNA sites with similar consensus sequences located in their common intergenic region (Fig. 6C).

We further confirmed the identified putative PtrR-binding site upstream of the sad genes in E. coli and conducted genome-wide mapping of other PtrR-binding sites using the ChIP-exo method. To identify in vivo PtrR binding sites, E. coli was grown under glucose as the carbon source in the M9 minimal media. A total of ninePtrR-binding sites were detected in these experiments. PtrR binds in the promoter regions of the fnrS, and ptrR/sad genes. The experimentally identified 50-bp PtrR-binding region at sad.ptrR genes contains the conserved palindromic DNA motif identified via phylogenetic footprinting (Fig. S8). Comparison of this DNA motif with eight other regions containing experimentally mapped PtrR-binding regions did not reveal significant sequence similarity except for the PtrR-binding area at fnrS, which shares a common consensus with the identified DNA motif at sad.ptrR. We created multiple alignments of the upstream DNA sequences of closely related species with the beginning of the ptrR gene was deleted under the starvation conditions (Fig. S9); a decrease in the growth.

Table 2. Differentially expressed genes revealed by RNA-Seq ygfB deletion mutant strain and wild type E. coli strains during growth in M9 medium with glucose as the primary carbon source and 7 mM L-threonine as supplement.

| Gene name | Gene function | Base mean | log² fold change |
|-----------|---------------|-----------|------------------|
| b1531     | marA          | 33.0      | 5.3              |
| b2021     | hisC          | 960.3     | 5.3              |
| b2031     | ydY           | 133.1     | 5.2              |
| b1926     | flagellar biosynthesis protein FlIT | 21.2 | 5.2 |
| b4307     | yjiQ          | 2.1       | 5.2              |
| b2024     | hisA          | 608.7     | 5.2              |
| b1889     | motB          | 409.8     | 5.2              |
| b4154     | fdaA          | 2376.0    | 5.2              |
| b1112     | bhsA          | 3.5       | 5.2              |
| b0621     | ducA          | 120.9     | 5.1              |
| b1878     | flkE          | 27.4      | 5.1              |
| b2020     | hisD          | 1390.9    | 5.1              |
| b1945     | flm           | 174.1     | 5.1              |
| b1885     | tap           | 1641.7    | 5.0              |
| b2022     | hisB          | 950.7     | 5.0              |
| b0297     | eaeH          | 1.9       | 5.0              |
| b3476     | nikA          | 126.7     | 5.0              |
| b1080     | fgl           | 263.1     | 5.0              |
| b1742     | ves           | 111.3     | 4.9              |
| b1200     | dhaK          | 127.4     | 4.4              |
| b3634     | exuD          | 40.9      | 2.6              |
| b2025     | hisF          | 1087.6    | 4.9              |
**Figure 4.** The systems approach for the function of the transcription factor YiaU. (A). The genome-wide binding of YiaU across the genome. (B). Clusters of Orthologous Groups (COGs) were enriched among the differentially expressed genes between the wild type BW25113 and yiaU mutant strains. (C). The phenotypes of the E. coli BW25113 and yiaU knockout strains in the Biolog plate PM12B, measured in RPMI_10LB medium for oxacillin at 4 different doubling concentrations. yiaU mutant strain (left panel) had a negative phenotype at the highest oxacillin concentration (8X) PM12B microarray (yellow line). The other antibiotics tested at 4 different concentrations are penicillin G, tetracycline, carbenicillin, penimepcycline, polymyxin B, paromomycin, vancomycin, d,l-serine hydroxamate, sisomycin, sulfamethazine, novobiocin, 2,4-diamino-6,7-dilsopropyl-pteridine, sulfadiazine, benzethonium chloride, tobramycin, 5-fluoro-orotate, spectinomycin, l-aspartic-b-hydroxamate, spiramycin, rifampicin, dodecyl trimethyl ammonium bromide. (D). The growth measurement of the wild type and yiaU mutant strains under different conditions. Left panel: M9 glucose with l-threonine. Right panel: M9 glucose with l-threonine and 0.3 M NaCl. (E) Predicted structure and YiaU regulation of waa operon, yiaT and adeP in E.coli BW25113. The differentially expressed genes are shown by a red arrow. The waa and gltBD operon promoters are shown by a black arrow.

**Table 3.** Differentially expressed genes revealed by RNA-Seq of a yiaU knockout and wild type E. coli strains during growth in M9 medium supplemented with 7 mM l-threonine.
genes involved in resistance to antibiotics, oxidative stress, organic solvents, and heavy metals. We tested E. coli to semialdehyde dehydrogenase, GlsB (YneH), glutaminase. GadAB—two glutamate decarboxylase isoforms, GabD, Sad (YneI)—succinate-semialdehyde dehydrogenase, GlsB (YneH), glutaminase. GadAB—two glutamate decarboxylase isoforms, GabD, Sad (YneI)—succinate-semialdehyde dehydrogenase, GlsB (YneH), glutaminase. GadAB—two glutamate decarboxylase isoforms, GabD, Sad (YneI)—succinate-semialdehyde dehydrogenase, GlsB (YneH), glutaminase.

Figure 5. The experimental validation of the transcription factor YneJ (PtrR). (A) Overview of two alternative pathways of putrescine utilization in E. coli. PtrR-regulated genes are shown in yellow boxes. Transporters are shown in blue. Abbreviations: GABAld – gamma-aminobutyraldehyde, PatA—putrescine aminotransferase, PatD—gamma-aminobutyraldehyde dehydrogenase, YneJ (Sad)—succinate-semialdehyde dehydrogenase, YneI (Sad)—succinate-semialdehyde dehydrogenase, GlsB (YneH), glutaminase. GadAB—two glutamate decarboxylase isoforms, GabD, Sad (YneI)—succinate-semialdehyde dehydrogenase, GlsB (YneH), glutaminase. GadAB—two glutamate decarboxylase isoforms, GabD, Sad (YneI)—succinate-semialdehyde dehydrogenase, GlsB (YneH), glutaminase. GadAB—two glutamate decarboxylase isoforms, GabD, Sad (YneI)—succinate-semialdehyde dehydrogenase, GlsB (YneH), glutaminase.

The phenotype microarray using Biolog PM2A was tested under the microaerobic conditions for the ptrR mutant phenotype and the E. coli WT BW25113 strain. The phenotype using Glu as the energy/nitrogen source was minimal when D-glucosamine or dihydroxyacetone was the carbon source. The ptrR mutant in growth/respiration with glycine, l-ornithine, or gamma-hydroxybutyrate was observed using M9 medium with l-glutamate as the sole nitrogen source. Phenotypes for the ptrR mutant with d-tagatose, oxalamalic acid, gamma-hydroxybutyrate, glycine, and l-alaninamide were observed under the same conditions with Glu as a supplement (Fig. S10). The regulatory effect of PtrR during aerobic growth with putrescine/Glu as the nitrogen source was detected for M9 medium with glycerol as the primary carbon source. The growth of BW25113 (WT) as well as ptrR, yneH (glsB) null mutant strains are shown in Fig. 5B-C. The E. coli WT strain had a longer lag-phase compared to the ptrR mutant. A growth deficiency for a glsB mutant was observed under the same conditions, suggesting a functional relationship between GlsB (YneH) and Sad, encoding genes conserved in genome clusters of E. coli MG1655 strain and adapted MG1655 derivatives (iModulonDB, PRECISE2). The activation of the sad and glsB promoter at the ptrR mutant strain was detected.

PtrR-dependent regulation during growth with Ptr/Glu as nitrogen sources and antibiotic resistance. The E. coli WT and ptrR mutant were grown aerobically in M9 medium with 20 mM Glu and Ptr as nitrogen sources and 0.2% glycerol. To determine the effect of the ptrR deletion mutation, the cells were collected at the log-phase, and total mRNA was purified (see Materials and methods). PusU and PusUADE, SodB, and two copper related transport systems’ mRNAs were increased in the ptrR mutant strain (Table 5). SodB (superoxide dismutase) mRNA was increased in ptrR mutant and SodB produced H$_2$O$_2$. The copA and cus operons are regulated by the CusSR and HprRS system. H$_2$O$_2$ is the effector for HprRS and likely has a transcriptional effect for the copA and cus system.

Antibiotic resistance induced by ptrR mutation in E. coli BW25113 was detected. We propose that PtrR negatively controls the Fnr small RNA, which is involved in regulation of MarA mRNA. MarA is a global regulator of E. coli genes involved in resistance to antibiotics, oxidative stress, organic solvents, and heavy metals. We tested the ptrR mutant and wild type E. coli strains for antibiotic resistance using the Biolog plate 11C11. Since FnrS is under positive control of the global anaerobic regulator Fnr, E. coli was grown under microaerobic conditions. The ptrR mutant showed increased resistance to high concentrations of demeclocycline, a tetracycline group antibiotic, which survived after 42 h, in contrast to the wild type. We also detected the increased resistance of
The systems approach for the function of the transcription factor PtrR. (A) The zoom-in of the PtrR-binding site at the promoter region of the ptrR (yneJ) and sad genes. Location of sigma-H and sigma-E promoters is from the EcoCyc database. (B) Fluorescent polarization assay of PtrR binding to the predicted operator site at sad gene. PhrR protein from Halomonas was used as a negative control. (C) The phylogenetic tree of PtrR orthologous proteins and predicted PtrR-binding motifs in E. coli and related genomes of Enterobacteria and Pseudomonas spp. The maximum likelihood phylogenetic tree was constructed using RAxML. The distinct genomic context of ptrR genes from two major tree branches (groups of PtrR orthologs) is shown by arrows with the following colors: black (ptrR regulator), yellow (sad for succinate semialdehyde dehydrogenase), green (yneJ for glutaminase), pink (mcp for methyl-accepting chemotaxis protein), and blue (mfs for putative MFS-family transporter), while the predicted PtrR-binding sites are shown by black dots. Sequence logos of predicted DNA binding sites of PtrR from each of the two groups of analyzed species were constructed using WebLogo 2.0.

Discussion
In this study, we applied a systems approach to characterize the transcriptional responses of seven putative LTFs: YbdO (CitR), YbeF, YbdD, YcaN, YiaU (LpsR), YgfI (DhfA), and YneJ (PtrR) (Table 6). The transcriptional response for the deletion of each LTF had been detected by RNAseq in M9 minimal medium supplemented by 7 mM l-threonine for all yTFs, except PtrR. The transcriptional analysis for the ptrR deletion mutant was detected in the M9 medium with Ptr and/or Glu as nitrogen source. For LTFs, conserved adjacent genes had been shown to be differentially expressed. For example, gene clusters encoding ybdNM and citrate utilization genes citCDEF (citrate lyase) were detected as transcriptionally regulated in the citR deletion mutant. The CitR DNA-binding site upstream of citR has been predicted and confirmed by ChIP-exo assay, suggesting autoregulation. CitR has been shown to be important for the growth in minimal medium supplemented by citrate at acidic conditions, suggesting citrate lyase regulation microaerobically. Additionally, flagella biosynthesis genes (FlhDC regulon) were differentially expressed in the mutant. The citR mutant in E. coli BW25113 fitness phenotype had been previously shown for motility in LB medium. Additionally, CitR is important for E. coli BW25113 fitness in minimal medium with glycolate as the carbon source (fit.genomics.lbl.gov), and D-glycine as the nitrogen source. The phenotype for formate and l-glutamate utilization citR deletion mutant was detected microaerobically (Fig. 7). YbeF is conserved in the gene cluster with citrate lyase encoding genes. The ybeF deletion leads to lrha gene downregulation and upregulation of FlhDC regulated genes and, accordingly, the FlhDC iModulon.

The YgfI (DhfA) DNA-binding site upstream of the dhaKLM operon for dihydroxyacetone phosphotransferase was detected by ChIP-exo. The transcriptome analysis shows that DhfA was important for regulation of dhaKLM, pfIB, hybcDEF, narZYWV and adhB. The dhfA mutant growth phenotype using glycerol as a carbon source had been detected (Fig. 3C). The common DNA-binding motif upstream of the dhaik, pfIB, adhE, hybc, and narZ genes was found, but future experiments to confirm DhfA binding to the predicted DNA-binding sites are essential (Table 6). The PfIB and hybcDEF encoded hydrogenase are involved in pyruvate and Thr utilization as energy source (Fig. 3F). The dhfA mutant growth deficiency on glucose as the carbon source in minimal medium at microaerobic conditions had been shown, but supplementation by Thr reduced the growth phenotype (Fig. S7), suggesting DhfA dependent pfIB and hybcDEF activation important for anaerobic metabolism.

The ptrR mutant was detected microaerobically using glycerol as a carbon source (Fig. S11). However, with other antibiotics tested, no significant difference in growth of the mutant and wild type strains was observed.

Figure 6. The systems approach for the function of the transcription factor PtrR. (A) The zoom-in of the PtrR-binding site at the promoter region of the ptrR (yneJ) and sad genes. Location of sigma-H and sigma-E promoters is from the EcoCyc database. (B) Fluorescent polarization assay of PtrR binding to the predicted operator site at sad gene. PhrR protein from Halomonas was used as a negative control. (C) The phylogenetic tree of PtrR orthologous proteins and predicted PtrR-binding motifs in E. coli and related genomes of Enterobacteria and Pseudomonas spp. The maximum likelihood phylogenetic tree was constructed using RAxML. The distinct genomic context of ptrR genes from two major tree branches (groups of PtrR orthologs) is shown by arrows with the following colors: black (ptrR regulator), yellow (sad for succinate semialdehyde dehydrogenase), green (yneJ for glutaminase), pink (mcp for methyl-accepting chemotaxis protein), and blue (mfs for putative MFS-family transporter), while the predicted PtrR-binding sites are shown by black dots. Sequence logos of predicted DNA binding sites of PtrR from each of the two groups of analyzed species were constructed using WebLogo 2.0.
Table 4. Differentially expressed genes revealed by RNA-Seq of a ptrR knockout and wild type E. coli strains during growth in M9 medium with l-glutamate and putrescine as nitrogen sources and glycerol as the primary carbon source.

| Locus tag | Gene name | Gene function | P value | Base mean | log2 fold change |
|-----------|-----------|---------------|---------|-----------|-----------------|
| b0123     | cueO      | Blue copper oxidase CueO | 1.53E−04 | 178       | 1.8             |
| b0484     | cepA      | Copper-exporting P-type ATPase | 3.00E−05 | 521       | 1.8             |
| b0570     | cusS      | Sensor histidine kinase CusS | 4.39E−04 | 112       | 2.2             |
| b0571     | cusR      | Transcriptional regulatory protein | 2.97E−05 | 181       | 2.5             |
| b0572     | cuxC      | Cation efflux system protein CuxC | 1.42E−05 | 949       | 7.8             |
| b0574     | cuxB      | Cation efflux system protein CuxB | 3.81E−05 | 208       | 6.0             |
| b0575     | cuxA      | Cation efflux system protein CuxA | 6.52E−05 | 281       | 4.8             |
| b0778     | BioD1     | ATP-dependent dethiobiotin synthetase BioD1 | 1.11E−04 | 126       | −1.4            |
| b1297     | puuA      | Gamma-glutamyl/putrescine synthetase | 4.64E−07 | 706       | 1.9             |
| b1298     | puuD     | Gamma-glutamyl-gamma-aminobutyrate hydrolase PuuD | 6.21E−05 | 337       | 1.9             |
| b1299     | puuR      | HTH-type transcriptional regulator PuuR | 9.24E−05 | 113       | 1.3             |
| b1302     | puuE      | 4-aminobutyrate aminotransferase PuuE | 3.16E−04 | 161       | 1.6             |
| b1495     | nuoK      | NADH-quinone oxidoreductase subunit K | 4.59E−07 | 73        | −1.9            |
| b1496     | yddA      | Inner membrane ABC transporter ATP-binding protein YddA | 1.25E−06 | 63        | −2.3            |
| b1526     | yncT      | Transcriptional regulator YncT | 9.41E−06 | 26        | −6.2            |
| b1596     | ynpM      | Inner membrane transport protein YnpM | 3.06E−04 | 221       | −1.5            |
| b1656     | sodB      | Superoxide dismutase [Fe] | 5.46E−05 | 323       | 4.8             |
| b1717     | rpmI      | SOS ribosomal protein L5 | 3.57E−05 | 55        | 2.4             |
| b1886     | tar       | Methyl-accepting chemotaxis protein II | 4.20E−05 | 298       | 1.4             |
| b1889     | motB      | Motility protein B | 3.08E−04 | 61        | 1.6             |
| b2094     | gatA      | PTS system galactitol-specific EIIA component | 6.09E−05 | 1719      | −1.4            |
| b2106     | rnaA      | Nickel/cobalt efflux system RnaA | 5.47E−07 | 323       | −1.5            |
| b3858     | yibD      | Protein YibD | 1.29E−06 | 42        | 2.6             |
| b3938     | metF      | Met repressor | 3.84E−04 | 191       | 1.4             |
| b4142     | groS      | 10 kDa chaperonin | 1.40E−04 | 197       | 1.9             |
| b4207     | fkbB      | FKBP-type peptidyl-prolyl cis–trans isomerase | 2.03E−05 | 539       | 1.5             |
| b4314     | fimA      | Type-1 fimbrial protein, A chain | 3.46E−04 | 1118      | 1.4             |

Table 5. Differentially expressed ptrR adjacent genes revealed by RNA-Seq ptrR(yneJ) deletion mutant strain and wild type E. coli strains during growth in M9 medium with glucose as the primary carbon source and 20 mM l-glutamate as nitrogen source.

| Locus tag | Base mean | log2 fold change | p value | Gene name |
|-----------|-----------|-----------------|---------|-----------|
| b1525     | 222.2     | 1.32            | 0.022   | yneJ      |
| b1526     | 26.1      | −6.69           | 0.0003  | yncT      |
| b1527     | 14.1      | 2.78            | 0.032   | yncK      |

Table 6. Summary of the yTFs newly characterized in this study. Abbreviation: LPS-lipopolysaccharide.
Figure 7. The systems approach for the phenotype detection of the transcription factors ybdO, ycaN and ygfI deletion mutants. The phenotype microarray (Biolog PM1 plate) (A. 12 h and B. 24 h growth) in M9 medium (95 carbon sources for ybdO and ygfI, ycaN deletion mutant strains compared to E. coli BW25113 (wild type, WT) strain. The carbon sources in PM1 plate: l-arabinose, N-acetyl-glucosamine, succinate, D-galactose, L-aspartate, L-proline, D-alanine, D-trehalose, D-mannose, D-serine, acetate, D-fructose, L-rhamnose, L-lactate, D-xylene, D-sorbitol, L-fucose, D-glucuronate, D-glucuronate, D-glycerol-P, D-glucose, D-melibiose, Lactose, maltose, uridine, L-glutamine, adenosine, L-glutamate, adenosine, L-serine, L-threonine, etc.

We suggest re-name YiaU to LpsR, lipopolysaccharide biosynthesis regulator. ChiP-exo detected multiple LpsR DNA-binding sites (Fig. 4A). The RNAseq transcriptomic analysis and ChiP-Exo (LpsR DNA-binding) additionally detected direct regulation of the waaPSBOJYZU operon gltB and adeP genes, suggesting LpsR relationship to glutamate metabolism. LpsR deletion mutant transcriptomic analysis suggested the regulation of yiaT, the conserved adjacent gene to lpsR, which is divergently transcribed and yeaK, encoding deacetylase for mischarged aminoacyl-tRNA (L-serine, L-threonine) (Table 3, Fig. 4D). The supplement in minimal medium could lead to mischarged tRNA and LpsR is important for the yeaK regulation in the presence of Thr. The lpsR deletion leads to increased sensitivity to oxacillin in RPMI_10LB medium microaerobically and LpsR was found to have the effect on the biofilm formation previously.

The ybbH adjacent gene ybhH conserved in enterobacterial genomes was upregulated in the ybbD deletion mutant. The ybhH, ybhl, and ybbD genes are conserved adjacent genes and potentially regulated by Nac (EcoCyc). YbhH is the putative tricarboxylate transporter, homologous to 2-oxoglutarate/malate translocator, (id. 35%) (Fig. S8). We detected the ybbD deletion strain growth phenotype in the M9 minimal medium with glycerol (carbon source), supplemented by L-malate (Fig. S9), but not in the absence of L-malate, suggesting that the de-repressed ybbH and ybhH genes are probably involved in L-malate utilization (Table 6). We detected that PtrR (YneJ) is the transcriptional regulator for Sad and the small RNA, FnrS. PtrR was predicted to be a repressor of the fnrS gene, encoding a small regulatory RNA (Table 6). We demonstrated PtrR binding to the predicted DNA-binding site. According to RNA-Seq data, PtrR is a repressor for sad under the nutrient limitation-stress conditions. The ptrR gene deletion effect was shown by growth phenotype (aerobically) and phenotype microarray analysis (micro-aerobically).

PtrR-mediated regulation appears to be important forPtr utilization as an energy source. A pleiotropic effect of the PtrR-dependent regulation of the sad gene under nitrogen/carbon starvation has been investigated and discussed. The known stress/starvation sigma σ5-controlled csiD-ygaF-gabDTP region is related to GABA utilization, while Sad is important for Ptr utilization.

Extracellular Ptr alters the OmpF porin charge and pore size, resulting in partial pore closure and a consequent decrease in outer membrane permeability. Our results demonstrated that PtrR is important for the growth of the E. coli BW25113 strain with Glu as the sole nitrogen source and glycerol as the carbon source and resistance to the tetracycline group of antibiotics (i.e., demeclocycline and chlorotetracycline), but not to chloramphenicol, erythromycin, and other antibiotics. PtrR is potentially important for the regulation of the highly conserved, anaerobically induced small RNA- fnrS, which is likely important for regulation under anaerobic
growth conditions. Interestingly, a ptrR mutant was shown previously to be resistant to bacteriophage lambda infection and we found that PtrR is potentially related to tetracycline resistance. ChiP-exo and RNAseq results have been analyzed, providing a hypothesis for the physiological functions of YneJ (tentatively re-named PtrR, putrescine related regulator), Ygfl (tentatively re-named DhfA, dihydroxyacetone phosphotransferase and formate utilization activator), and YbdO (tentatively re-named CtrR, citrate utilization related regulator).

The identification of the additional DNA-binding sites for Ygfl, YcaN, YiuU, YbeF; YneJ by gSELEX (genomic SELEX) method in the presence of Thr in the minimal medium possibly will provide additional information about novel LTFs transcriptional regulatory network. LTFs are not always expressed under laboratory growth conditions (for instance, see Ishihama et al. J. Bacteriol. 196, 2718–2727, 2014).

Taken together, the systems analysis of the E. coli BW25113 and MG1655 strains transcriptomic data, ChiP-exo DNA-binding data for LTF, and the regulated biochemical pathway reconstruction and fitness/phenotype of the LTF deletion mutants strains produce fruitful hypotheses for the yTF function prediction that is important for TRN reconstruction in E. coli.

Methods

RNA sequencing. The wild type BW25113 strain was grown as a control for the isogenic ptrR mutant strain. Pre-cultures were obtained by scraping frozen stocks and growing the cells in LB medium. Cells were washed twice with M9 medium and inoculated to an OD$_{600}$ of 0.05. The cells were collected at an OD$_{600}$ of 2.0; late-exponential phase) and were harvested using the Qiagen RNA-proteck bacteria reagent according to the manufacturer’s specifications. Pelleted cells were stored at – 80 °C, and after cell resuspension and partial lysis, they were ruptured with a beat beater; the total RNA was extracted using a Qiagen RNA purification kit. After total RNA extraction and subsequent ribosomal RNA removal, the quality was assessed using an Aglient Bioanalyser using an RNA 6000 kit. The data processing is described in Supplemental materials.

Protein purification. The PrtR-producing strain was grown overnight, re-inoculated into 50 mL of the fresh medium, and induced with 0.6 mM IPTG after an OD$_{600}$ of 0.6 was reached. The cells were harvested after 4 h and lysed, the cell pellet was resuspended in the lysis bufer. Rapid purification of recombinant proteins on Ni-nitrilotriacetic acid-agarose minicolumns was performed. The protein was refolded on a mini-column, and the buffer was changed to a buffer containing 0.1 M Tris–HCl, 0.1 M NaCl, 10 mM urea.

Fluorescent polarization assay. The purified PrtR protein and 10 nM fluorescently labeled DNA fragment (5′-ggggTTC TCG ATT CGT GAAggg-3′) were incubated in the assay mixture. The PrtR binding assay mixture (0.1 ml) contained Tris buffer, pH 7.5, 0.1 M NaCl, 10 mM MgSO$_4$, 5 mg/ml sperm DNA and 1uM of the fluorescently labeled predicted PrtR binding DNA fragment as well as 0–0.6 mM GABA. Then the PrtR protein (0–1.5 uM) was added to the assay mixture, and it was incubated for 1 h at 30 °C in the presence or the absence of GABA.

Targeted high-performance liquid chromatography. For organic acid and carbohydrate detection, samples were collected after 4 h for every 30–45 min. The filtered samples were loaded onto a 1260 Infinity series (Agilent Technologies) high-performance liquid chromatography (HPLC) system with an Aminex HPX-87H column (Bio-Rad Laboratories) and a refractive index detector and HPLC was operated using ChemStation software. The HPLC was run with a single mobile phase composed of HPLC grade water buffered with 5 mM sulfuric acid (H$_2$SO$_4$). The flow rate was held at 0.5 mL/minute, the sample injection volume was 10 uL, and the column temperature was maintained at 45 °C. The identities of compounds were determined by retention time comparison to standard curves of acetate, ethanol, glucose, lactate, pyruvate, formate and succinate. The peak area integration and resulting chromatograms were generated within ChemStation and compared to that of the standard curves to determine the concentration of each compound in the samples.

Phenotype microarray. The E. coli BW25113 wild type and ybdO, ygfl, ptrR mutant strains were grown overnight in M9 glucose medium, washed with M9 medium (PM1, PM2) or RPMI with 10% LB (RPMI LB) for PM12B and inoculated as recommended to the Omnolog plates PM1, PM2, PM11C or PM12B, for the antibiotic resistance measurements at 37 °C. The experiments were repeated two times.

Received: 16 November 2021; Accepted: 14 April 2022
Published online: 04 May 2022

References

1. Gao, Y. et al. Unraveling the functions of uncharacterized transcription factors in Escherichia coli using ChiP-exo. Nucleic Acids Res. 49, 9696–9710 (2021).
2. Rodionova, I. A. et al. Identification of a transcription factor, PunR, that regulates the purine and purine nucleoside transporter purC in E. coli. Commun. Biol. 4, 991 (2021).
3. Schellhorn, H. Faculty opinions recommendation of novel regulators of the csgD gene encoding the master regulator of biofilm formation in Escherichia coli K-12. In Faculty Opinions—Post-Publication Peer Review of the Biomedical Literature (2020). https://doi.org/10.3410/F.738307210.793578431.
4. Yamanaka, Y., Shimada, T., Yamamoto, K. & Ishihama, A. Transcription factor CcrC (YbhH) regulates a set of genes affecting the sensitivity of Escherichia coli against cephaloridine and chloramphenicol. Microbiology 162, 1253–1264 (2016).

5. Maddocks, S. E. & Oyston, P. C. F. Structure and function of the LysR-type transcriptional regulator (LTT) family proteins. Microbiology 154, 3609–3623 (2008).

6. Ishihama, A., Shimada, T. & Yamanaka, Y. Transcription profile of Escherichia coli: Genomic SELEX search for regulatory targets of transcription factors. Nucleic Acids Res. 44, 2058–2074 (2016).

7. Knapp, G. S. & Hu, J. C. Specificity of the E. coli LysR-type transcriptional regulators. PLoS ONE 5, e15189 (2010).

8. Lachnit, T., Bosch, T. C. G. & Deines, P. Exposure of the host-associated microbiome to nutrient-rich conditions may lead to dysbiosis and disease development—an evolutionary perspective. mBio 10, 0035519 (2019).

9. Rychel, K. et al. iModulonDB: A knowledgebase of microbial transcriptional regulation derived from machine learning. Nucleic Acids Res. 49, D112–D120 (2021).

10. Website. Price, Morgan (2020): The Fitness Browser: Genome-wide mutant fitness data for diverse bacteria (November 2020 release). figshare. Dataset. https://doi.org/10.6084/m9.figshare.13172087.v1.

11. Rodionova, I. A. et al. The uridylyltransferase GlnD and tRNA modification GTPase MnmE allosterically control poly-

12. Li, J. et al. Polymers disrupt the KaiABC oscillator by inducing protein denaturation. Molecules 24 (2019).

13. Latour, Y. L., Gobert, A. P. & Wilson, K. T. The role of polyamines in the regulation of macrophage polarization and function. Amino Acids 52, 151–160 (2020).

14. Publio, B. C., Moura, T. A., Lima, C. H. M. & Rocha, M. S. Biophysical characterization of the DNA interaction with the biogenic polyamine putrescine: A single molecule approach. Int. J. Biol. Macromol. 112, 175–178 (2018).

15. Tkachenko, A. G., Pozhdaeva, O. N. & Shumakov, M. S. Role of polymers in formation of multiple antibiotic resistance of Escherichia coli under stress conditions. Biochemistry 71, 1042–1049 (2006).

16. Nemoto, N. et al. Mechanism for regulation of the putrescine utilization pathway by the transcription factor PuuR in Escherichia coli K-12. J. Bacteriol. 194, 3437–3447 (2012).

17. Kuribara, S. et al. Gamma-GLUTamylputrescine synthetase in the putrescine utilization pathway of Escherichia coli K-12. J. Biol. Chem. 283, 19981–19990 (2008).

18. Kuribara, S., Kato, K., Asada, K., Kumagi, H. & Suzuki, H. A putrescine-inducible pathway comprising PuuE-YneI in which gamma-aminobutyrate is degraded into succinate in Escherichia coli K-12. J. Bacteriol. 192, 4582–4591 (2010).

19. Gao, Y. et al. Systematic discovery of uncharacterized transcription factors in Escherichia coli K-12 MG1655. Nucleic Acids Res. 46, 10682–10696 (2018).

20. Romiyo, V. & Wilson, J. W. Phenotypes, transcriptome, and novel biofilm formation associated with the yedl gene. Antonie Van Leeuwenhoek 113, 1109–1122 (2020).

21. Lehnen, D. et al. LrHA as a new transcriptional key regulator of flagella, motility and chemotaxis genes in Escherichia coli. Mol. Microbiol. 45, 521–532 (2002).

22. Gonzalez, R., Murakka, A., Dharmadi, Y. & Yudzani, S. S. A new model for the anaerobic fermentation of glycerol in enteric bacteria: Trunk and auxiliary pathways in Escherichia coli. Metab. Eng. 10, 234–245 (2008).

23. Lee, J.-H., Lee, K.-L., Yeo, W.-S., Park, S.-J. & Roe, J.-H. SoxRS-mediated lipopolysaccharide modification enhances resistance against multiple drugs in Escherichia coli. J. Bacteriol. 191, 4441–4450 (2009).

24. Yeo, S.-I., Jeong, J.-H., Yu, S.-N. & Kim, Y.-G. Crystallization and preliminary X-ray crystallographic analysis of YgjG from Escherichia coli. Acta Crystallogr Sect. F Struct. Biol. Cryst. Commun. 68, 1070–1072 (2012).

25. Schneider, B. L. & Reitzer, L. Pathway and enzyme redundancy in putrescine catabolism in Escherichia coli. J. Bacteriol. 194, 4080–4088 (2012).

26. Park, S. A., Park, Y. S. & Lee, K. S. Kinetic characterization and molecular modeling of NAD(P)(+)-specific succinate semialdehyde dehydrogenase from Bacillus subtilis as an ortholog YneI. J. Bacteriol. 184, 2621–2627 (1995).

27. Lee, S. et al. The 2-oxoglutarate/malate translocator of chloroplast envelope membranes: Molecular cloning of a transporter containing a 12-helix motif and expression of the functional protein in yeast cells. Biochemistry 34, 2621–2627 (1995).

28. Schneider, B. L. et al. The Escherichia coli gabDTPC operon: Specific gamma-aminobutyrate catabolism and nonspecific induction. J. Bacteriol. 184, 6976–6986 (2002).

29. Iyer, S. et al. Distinct mechanisms coordinate transcription and translation under carbon and nitrogen starvation in Escherichia coli. Nat. Microbiol. 3, 741–748 (2018).

30. Shimizu, K. Metabolic regulation and coordination of the metabolism in bacteria in response to a variety of growth conditions. In Bioreactor Engineering Research and Industrial Applications I 1–54 (2015). https://doi.org/10.1007/978-1-4939-2653-0_2.

31. Iyer, R. & Delcour, A. H. Complex inhibition of OmplP and OmplPc bacterial pores by polymyxins. J. Biol. Chem. 272, 18595–18601 (1997).

32. Durand, S. & Storz, G. Reprogramming of anaerobic metabolism by the FnrS small RNA. Mol. Microbiol. 75, 1215–1231 (2010).

33. Banzhal, W. et al. Evolution in Action: Past, Present and Future: A Festschrift in Honor of Erik D. Goodman (Springer, New York, 2020).

34. Rodionov, D. A., Gelfand, M. S., Mironov, A. A. & Rakhmaninova, A. B. Comparative approach to analysis of complex genomes: Multidrug resistance systems in gamma-proteobacteria. J. Microbiol. Microbiol. Biotechnol. 3, 319–324 (2001).

35. Ishihama, A. Prokaryotic genome regulation: Multifactor promoters, multistart regulators and hierarchic networks. FEMS Microbiol. Rev. 34, 628–645 (2010).

Author contributions
I.R., Y.G., M.S., H.L. and B.P. wrote the manuscript text and I.R., B.P. and D.R. prepared the figures, N.W., R.S., Z.Z. and Y.H. provide experimental analysis and experiments, H.L., D.R., J. M. provide bioinformatics analysis, Hyun Gyu Lim (H.L.).

Competing interests
The authors declare no competing interests.
