The PurR regulon in *Escherichia coli* K-12 MG1655

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ABSTRACT

The PurR transcription factor plays a critical role in transcriptional regulation of purine metabolism in enterobacteria. Here, we elucidate the role of PurR under exogenous adenine stimulation at the genome-scale using high-resolution chromatin immunoprecipitation (ChIP)-chip and gene expression data obtained under in vivo conditions. Analysis of microarray data revealed that adenine stimulation led to changes in transcript level of about 10% of *Escherichia coli* genes, including the purine biosynthesis pathway. The *E. coli* strain lacking the purR gene showed that a total of 56 genes are affected by the deletion. From the ChIP-chip analysis, we determined that over 73% of genes directly regulated by PurR were enriched in the biosynthesis, utilization and transport of purine and pyrimidine nucleotides, and 20% of them were functionally unknown. Compared to the functional diversity of the regulon of the other general transcription factors in *E. coli*, the functions and size of the PurR regulon are limited.

INTRODUCTION

Metabolism enables a cell to assimilate exogenous nutrients both for energy generation and for macromolecular synthesis. A set of metabolic pathways directs the biosynthesis and utilization of nucleotides that is critical for virtually every aspect of cellular life. Purine and pyrimidine nucleotides constitute a part of the nucleic acids, cofactors in enzymatic reactions, intracellular and extracellular signals, phosphate donors and the major carriers of cellular energy (1,2). Since the biosynthesis and utilization of nucleotides is demanding of cellular resources and cellular energy (1,2), purine metabolism is tightly controlled by feedback inhibition of enzyme activity by metabolites and by transcriptional regulation by DNA-binding proteins. The regulatory action of DNA-binding proteins is also modulated by the exogenous nutrients as stimuli. Therefore, there is great interest in not only elucidating the set of genes under regulation by the same stimuli (defined as a stimulon), but also identifying the collection of genes under regulation by the same regulatory protein (defined as a regulon). In the case of purine nucleotide metabolism in *Escherichia coli*, purine repressor (PurR) tightly regulates transcription of the enzymes involved in inosine 5'-monophosphate (IMP) biosynthesis and the conversion of IMP to adenosine monophosphate (AMP) and guanosine monophosphate (GMP)(2). The regulatory action of PurR on target genes is modulated by the binding of the small effector molecules [hypoxanthine (Hx) or guanine] and in effect endows PurR with the ability to affect transcriptional regulation (4). In other words, upon availability of purine nucleotides from the environment, the activity of PurR can be enhanced to repress the expression of target genes. However, little is known about *in vivo* PurR-binding events and their causal relationships with gene expression at the genome scale in the presence or absence of purine nucleotides. Such information is needed to reconstruct the PurR regulon and to understand purine metabolism.

The *E. coli* transcriptional regulatory network is believed to have a hierarchical topology with several global transcription factors (TFs) at the top-level (5–7). The global TFs were specified by the multiple functional categories of the genes regulated. By contrast, specific TFs repress their target genes to the same metabolic pathways or the same functional categories (6). Previously, PurR was classified into the group of general TFs; however, due to the lack of information on target genes in its regulon, the understanding of the role of PurR is limited. In particular, it is unclear whether its effects on *E. coli* metabolism are direct or indirect. If the effects are indirect, it is also unclear whether the indirect effects are made through other TFs or other metabolites.

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In this study, we obtain and integrate genome-scale data from chromatin immunoprecipitation (ChIP)–chip and gene expression profiling to elucidate the regulatory role of PurR at a genome scale. First, using changes in transcript levels on a genome scale, we defined the adenine stimulon from comprehensively established sets of genes differentially expressed in response to exogenous adenine. Second, we used the purR deletion mutant to determine the PurR-dependent genes affected by the deletion mutant. Third, we set out to comprehensively establish the PurR-binding regions on the E. coli genome experimentally to further elucidate any DNA sequence motif correlated with the PurR regulatory action. Fourth, we determined the regulatory action of PurR based on the causal relationships between the association of PurR and changes in transcript levels. In the end, the reconstruction of the regulatory network of PurR allows us to understand changes in transcript levels on a genome scale, we defined the adenine stimulon. The results show that the role of the PurR regulon as a part of the broader regulatory network of PurR allows us to understand the role of the PurR regulon as a part of the broader metabolic network. The results show that the role of PurR regulon is locally acting but its effect on the entire metabolism is critical in response to the exogenous purine stimulation.

MATERIALS AND METHODS

Bacterial strains and growth conditions

All strains used are E. coli K-12 MG1655 and its derivatives. The E. coli strain harboring PurR-8myc was generated as described previously (8). Deletion mutant (ΔpurR) was constructed by λ Red and FLP-mediated site-specific recombination system (9). Glycerol stocks of E. coli strains were inoculated into M9 minimal medium supplemented with 2 g/l glucose and cultured overnight at 37°C with constant agitation. The cultures were inoculated into 100 ml of the fresh M9 minimal medium in either the presence or absence of 100 μg/ml adenine and continued to culture at 37°C with constant agitation to mid-log phase.

Transcriptome analysis

Samples for transcriptome analyses were taken from exponentially growing cells. From the cells treated by 2 vol of RNAProtect Bacteria Reagent (Qiagen), total RNA was isolated using RNeasy kit (Qiagen) with DNaseI treatment in accordance with manufacturer’s instruction. AffymetrixGeneChip E. coli Genome 2.0 arrays were used for genome-scale transcriptional analyses. Complementary DNA (cDNA) synthesis, fragmentation, end-terminus biotin labeling and array hybridization were performed as recommended by Affymetrix standard protocol. Raw CEL files were analyzed using robust multi-array average for normalization and calculation of probe intensities.

ChIP and microarray analysis

To identify PurR-binding regions in vivo, we isolated the DNA bound to PurR protein by ChIP. Cultures at mid-log phase were cross-linked by 1% formaldehyde at room temperature for 25 min. After cell lysis and sonication, the cross-linked DNA-PurR complex was immunoprecipitated by using the specific antibody against myc-tag (9E10, Santa Cruz Biotech) and Dynabeads Pan Mouse IgG magnetic beads (Invitrogen) followed by stringent washings as described previously (10). After reversal of the cross-links by incubation at 65°C overnight, the samples were treated by RNaseA (Qiagen) and proteaseK (Invitrogen) and then purified with a PCR purification kit (Qiagen). Then, the amplified ChIP DNA samples were labeled and hybridized onto whole-genome tiled microarrays (Roche-NimbleGen).

Data analysis

To identify PurR-binding regions, we used the peak finding algorithm built into the NimbleScan™ software. Processing of ChIP–chip data was performed in three steps: normalization, IP/mock-IP ratio computation (log base 2), and enriched region identification. The log2 ratios of each spot in the microarray were calculated from the raw signals obtained from both Cy5 and Cy3 channels, and then the values were scaled by Tukey bi-weight mean. The log2 ratio of Cy5 (IP DNA) to Cy3 (mock-IP DNA) for each point was calculated from the scanned signals. Then, the bi-weight mean of this log2 ratio was subtracted from each point. Each log ratio dataset from triplicate samples was used to identify PurR-binding region using the software (width of sliding window = 300 bp). Our approach to identify the PurR-binding regions was to first determine binding locations from each data set and then combine the binding locations from at least five of the six data sets to define a binding region (11).

Motif searching

The PurR-binding motif analysis was completed using the MEME and FIMO tools from the MEME software suite (12). We first determined the proper binding motif and then scanned the full genome for its presence. The elicitation of the motif was done using the MEME program on the set of sequences defined by the PurR-binding regions. Using default settings, the previously determined PurR motif was recovered and then tailored to the correct size by setting the width parameter to 16 bp. We then used these motifs and the PSPM (position specific probability matrix) generated by MEME to rescanc the entire genome with the FIMO program.

RESULTS

Determination of gene expression changes to the exogenous adenine

To characterize the changes in gene expression with exposure to exogenous adenine, global transcriptome analyses were performed using DNA microarrays. Escherichia coli strain K-12 MG1655 (wild type) was grown in M9 medium supplemented by 100 μg/ml adenine. Samples were removed from the culture with and without adenine stimulation and used for the extraction of total RNA. Analysis of the DNA microarray data
demonstrated that exogenous adenine stimulation led to changes in transcript level of about 10% of *E. coli* genes (Supplementary Table S1). Those include the upregulation of 144 genes and downregulation of 255 genes with more than 2-fold expression change and *P*-value <0.05. As previously well described (2), all of the genes associated with the purine biosynthesis pathway were repressed by the addition of adenine. Among them, *purD* encoding phosphoribosylamine–glycine ligase showed the highest repression factor (43.89-fold) (Supplementary Table S2). Interestingly, adenine addition led to the high downregulation of several transporter genes, including *codB, xanP, yeeF* and *uraA* encoding cytosine transporter (34.52-fold), xanthine NCS2 transporter (20.12-fold), amino acid APC transporter (18.61-fold) and uracil NCS2 transporter (17.64-fold).

A significant portion of the genes downregulated by adenine addition is associated with the pyrimidine and amino acid biosynthetic pathways. In particular, the downregulation of genes comprising the arginine biosynthetic pathway clearly demonstrates that the purine metabolism links to the *in vivo* level of arginine in order to regulate the biosynthesis of deoxyribonucleic and ribonucleic acid (13). On the other hand, the highly induced genes by adenine stimulation were involved in other various cellular processes (Supplementary Table S2). Among them, *ydhC* encoding drug MFS transporter had the highest activation factor (193.55-fold). Of interest, two non-coding RNAs, *gcvB* and *rybB*, were induced by a factor of 34.37 and 5.92, respectively. Previous studies showed that GcvB enhances the ability of *E. coli* to survive low pH by upregulating the levels of alternate sigma factor, RpoS (14). Another alternate sigma factor, RpoE-dependent RybB, regulates the synthesis of major porins in *E. coli* (15). Among genes in purine salvage pathways, *add* encoding adenosine deaminase was induced by adenine as well (17.61-fold) (2). The genes in thiamine and biotin biosynthesis pathways were also induced by the exogenous adenine. These observations demonstrate that a large number of the downregulated genes related with purine and pyrimidine biosynthesis and transport, arginine biosynthesis and ATP synthesis coupled with proton transport form a major portion (~64%) of the adenine stimulon.

**PurR-dependent transcriptome response to the exogenous adenine**

Next, we studied the global response of a *purR* deletion mutant to adenine stimulation to identify PurR-dependent genes (Supplementary Table S1). To address this issue, we isolated total RNA from the isogenic *purR* deletion mutant during exponential growth phase and hybridized the cDNA obtained from the total RNA onto Affymetrix whole-genome tiling microarrays. A comparison of the gene expression levels between cells grown in the presence and absence of the PurR protein in response to the exogenous adenine revealed that a total of 56 genes exhibit differential expression with more than 2-fold change and a false discovery rate (FDR) value <0.05 (*P*-value = 0.0056) from analysis of variance (ANOVA) analysis (Supplementary Table S3).

Nineteen genes (~34%) showed increased transcript levels in response to the exogenous adenine due to regulation by PurR (Supplementary Table S3). None of these 19 genes has been previously reported to be directly regulated by PurR. On the other hand, transcription of the 37 genes (~66%) was repressed by PurR. Eleven genes of the IMP (inosine 5'-monophosphate) biosynthetic pathway from PRPP (5-phosphoribosyl-1-pyrophosphate) clustered into this group. It has been previously determined that 10 of them were directly repressed by the PurR protein (16–24). Eight genes in pyrimidine biosynthesis and transport pathways were directly or indirectly regulated by the PurR protein, that include *carA, carB, pyrB, pyrL, pyrC, pyrD, codA* and *codB*. It has been experimentally determined that four of them (*carA, carB, pyrD* and *pyrC*) were directly repressed by the PurR (25–29). Transcription of *yieG* (~2.91-fold), *xanP* (~30.82-fold), and *uraA* (~2.04-fold) encoding adenine, xanthine and uracil transporter, respectively, were also affected by the purR deletion, indicating direct or indirect regulatory effect of the PurR protein on the adenine and uracil transport systems (30). Transcriptional repression of genes in arginine biosynthesis pathway (*argA, argB* and *argC*) is potentially mediated by the PurR protein. Interestingly, acid stress response genes (*hdeB, hdeA* and *hdeD*) decreased expression in a *purR* deletion mutant. Consistent with this observation, the level of messenger RNA (mRNA) transcript of *gadY*, a regulatory small RNA that is highly upregulated by low pH (31), was affected by the purR deletion. A hallmark of the *E. coli* response to the exogenous adenine is the rapid and strong repression of a set of genes in purine biosynthesis pathway. The observed repression of all of these genes in the wild-type strain, but not the *purR* deletion mutant, provided an internal validation of the microarray experiment.

**Genome-wide identification of PurR regulon**

PurR-binding regions have been characterized by *in vitro* DNA-binding experiments and mutational analysis; however, direct analysis of *in vivo* PurR binding is not available. We thus employed the ChiP coupled with microarrays (ChiP-chip) approach to determine the *in vivo* PurR-binding regions in *E. coli* cells under either the presence or the absence of exogenous adenine (Figure 1).

We performed a hybridization of the immunoprecipitated DNA (Cy5 channel) and mock immunoprecipitated DNA (Cy3 channel) onto the high-resolution whole-genome tiling microarrays, which contained a total of 371,034 oligonucleotides with 50-bp tiles overlapping every 25 bp on both forward and reverse strands (11,32). The normalized log2 ratios obtained from the hybridization identify the genomic regions enriched in the IP-DNA sample compared with the mock IP-DNA sample and thereby represent a genome-wide map of *in vivo* interactions between PurR protein and *E. coli* genome (Figure 1A). Using a peak finding algorithm, 35 and 13 unique and reproducible PurR-binding regions were identified from the
hybridizations in exponential phase in the presence and absence of adenine, respectively (Table 1).

The genome-wide PurR-binding maps obtained from two different conditions, i.e. exponential growth phase in the presence and the absence of exogenous adenine, indicated that the PurR association on the *E. coli* genome is dramatically sensitive to the addition of adenine. For instance, PurR occupancy for the promoter regions of *carAB*, *purC*, and *purMN* transcription units showed a great differential ratio between those two conditions (Figure 1B, Table 1). At the previously characterized PurR-binding promoter regions of *pyrD*, *purB*, *purR*, *cvpA-purf-ubiX*, *guaBA*, *purL* and *purHD*, we only observed the PurR-binding in the presence of exogenous adenine. Only 37% of binding sites (13 of 35) overlapped under the conditions in the absence and presence of exogenous adenine, and 62% of binding sites (22 of 35) were found in the presence of exogenous adenine (Figure 1C). Adenine can be converted to IMP through the intermediate formation of adenosine, inosine, and Hx catalyzed by purine nucleoside phosphorylase (*deoD*) and adenosine deaminase (*add*) (2). Thus, this observation indicates that the addition of exogenous adenine increased in the intracellular level of Hx, which led to the formation of the PurR–Hx complex that functions in transcriptional regulation (4). A total of 22 new PurR-binding regions were identified in this study, whose roles were involved in various cellular processes (Table 1). Prior to this study, 15 PurR-binding regions had been characterized by DNA-binding experiments *in vitro* and mutational analysis *in vivo*, 87% (13 of 15) of which were identified in this study (transcription units in bold characters in Table 1). The exceptions were *pyrC* and *glnB* promoters, whose cellular functions are related to the pyrimidine biosynthesis and nitrogen metabolism, respectively. It is unclear why those PurR-binding regions were missed from our analysis.

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**Figure 1.** Genome-wide distribution of PurR-binding regions. (A) An overview of PurR-binding profiles across the *E. coli* genome at exponential growth phase in the presence (blue) or absence (red) of exogenous adenine. Black and white dots indicate previously known and newly found PurR-binding regions, respectively. (B) Determination of genuine PurR-binding regions on the selected regions. Promoter regions of *carAB*, *purC*, *yfgO/yfgC* and *purM* are occupied by PurR at exponential state. The peak height of the identified PurR-binding region is the log 2 enrichment ratio calculated from Cy5 (IP DNA) and Cy3 (mock IP DNA) signal intensity of the probe corresponding to the identified region. (C) Overlaps between PurR-binding regions of exponential phase in the presence (blue) and absence (red) of adenine. (D) Sequence logo representation of the PurR–DNA binding profile. (E) Comparison of ChIP–chip results and gene expression profiles.
We next assessed the locations of the PurR-binding regions against the current annotated genome information (11). The PurR-binding regions were observed only within intergenic (i.e., promoter and promoter-like) regions. Therefore, there exists a strong preference for the PurR-binding target to be located within the noncoding intergenic regions, similar to that observed for Lrp-binding sites (32). To identify common DNA sequence motifs of the PurR-binding regions, we used the MEME suite tool (12). The sequences of PurR-binding regions were used to generate the position specific probability matrix and to rescan the entire genome with the FIMO program. We then analyzed only those sites which were located in the PurR-binding regions and fell below a stringent cut-off (P-value < 0.0001). This revealed a total of 28 conserved sequences spread across 35 binding regions (Table 1). The identified sequence motif (ACGNAACGTTTGCNT) was consistent with the previously characterized 16-bp palindromic binding site of the PurR (Figure 1D) (2). Based on the fact that the increase in the intracellular adenine levels enhances PurR binding to its DNA targets and the coverage of the known binding regions in our data, we concluded that PurR-binding regions identified here are bona fide binding sites.

### Table 1. Genome-scale identification of PurR-binding regions

| Start | End | WA | WOA | Transcription unit | Motif position | Consensus sequence |
|-------|-----|----|-----|-------------------|----------------|-------------------|
| 28951 | 29929 | 2.08 | 0.70 | carA-carB | 29415–29430 | TGGAAAACGTTTCGCC |
| 169607 | 170394 | 1.00 | 0.73 | fluC-fluAD-fluB | 170015–170030 | AGCCAAAGGCTTTGGCC |
| 354056 | 354323 | 1.41 | | codB-codA | 354064–354079 | AGCCAAAGGCTTTGCTT |
| 430925 | 431911 | 2.08 | 1.48 | Txx | 431376–431391 | AGCTAATGCGATGCTT |
| 552127 | 552626 | 2.08 | 0.86 | purE-purK | 552395–552410 | AGCCAAAGGCTTTGCTT |
| 1003618 | 1004017 | 1.01 | | pyrD | 1003899–1003904 | CGGAAAACGTTTCGC |
| 1190891 | 1191196 | 1.90 | | purB | 1191010–1191025 | AGCTAATGCGATGCTT |
| 1735528 | 1736095 | 1.99 | | purR | 1735808–1735823 | AGCTAATGCGATGCTT |
| 1766545 | 1767225 | 1.08 | | ydhJ-ydhK | 1767502–1767517 | AGCTAATGCGATGCTT |
| 1928502 | 1929233 | 2.00 | 1.02 | pyrT | 1928822–1928837 | AGCTAATGCGATGCTT |
| 2226706 | 2227311 | 1.13 | | yohP | 2227688–2227693 | AGCTAATGCGATGCTT |
| 2428603 | 2428966 | 1.77 | | cvpA-purF-ubiX | 2428842–2428857 | AGCTAATGCGATGCTT |
| 2439185 | 2439884 | 0.97 | | fabB-mmnmC | 2439210–2439225 | AGCTAATGCGATGCTT |
| 2594676 | 2596862 | 2.08 | 1.52 | purC | 2595794–2595809 | AGCTAATGCGATGCTT |
| 2617278 | 2614177 | 1.18 | | yfgO-yfgC | 2613843–2613858 | AGCTAATGCGATGCTT |
| 2618847 | 2619484 | 2.08 | 0.98 | purM-purN | 2619140–2619155 | AGCTAATGCGATGCTT |
| 2622091 | 2623533 | 0.84 | | gnuB-gnuA | 2632146–2632161 | AGCTAATGCGATGCTT |
| 2683277 | 2683995 | 2.08 | 1.13 | glyA | 2683648–2683663 | AGCTAATGCGATGCTT |
| 2693490 | 2693714 | 1.88 | | purL | 2693642–2693657 | AGCTAATGCGATGCTT |
| 2925514 | 2926070 | 2.08 | 0.83 | sdcA-sdcB | 2925844–2925859 | AGCTAATGCGATGCTT |
| 3013992 | 3014047 | 1.00 | | yfgK-ssnA | 3013768–3013783 | AGCTAATGCGATGCTT |
| 3028215 | 3029251 | 2.08 | | yfgT-yfgS | 3028661–3028676 | AGCTAATGCGATGCTT |
| 3048803 | 3049539 | 1.06 | | gevT-gevH-gevP | 3048572–3048593 | AGCTAATGCGATGCTT |
| 3396153 | 3396727 | 1.26 | | ydhE | 3397104–3397119 | AGCTAATGCGATGCTT |
| 3490230 | 3490492 | 1.08 | | ppiA/sgA | 3490704–3490719 | AGCTAATGCGATGCTT |
| 3550769 | 3551100 | 0.86 | | malT | 3551001–3551016 | AGCTAATGCGATGCTT |
| 3607049 | 3607298 | 1.39 | | yibQ | 3607081–3607096 | AGCTAATGCGATGCTT |
| 3630018 | 3663864 | 1.56 | 1.06 | gudX | 3663653–3663668 | AGCTAATGCGATGCTT |
| 3826187 | 3827555 | 2.08 | 1.36 | xanP | 3826900–3826915 | AGCTAATGCGATGCTT |
| 3889003 | 3889940 | 2.08 | 1.52 | mtlI | 3889723–3889738 | AGCTAATGCGATGCTT |
| 3894397 | 3895190 | 2.08 | | yieG | 3894732–3894747 | AGCTAATGCGATGCTT |
| 4205612 | 4470784 | 1.75 | | purH-purD | 4205663–4205678 | AGCTAATGCGATGCTT |
| 4275855 | 4276485 | 2.08 | | rjyA/rjyD | 4276230–4276245 | AGCTAATGCGATGCTT |
| 4402483 | 4402483 | 1.19 | | pyrL-pyrB-pyrI | 4402483–4402483 | AGCTAATGCGATGCTT |
| 4470473 | 4470609 | 1.43 | 0.58 | pyrL-pyrB-pyrI | 4470609–4470614 | AGCTAATGCGATGCTT |

**Genome-scale determination of causal relationship**

Currently, a total of 22 genes have been characterized as members of PurR regulon to be directly repressed by PurR (33). From our ChIP-chip analyses, we significantly expanded the size of the PurR regulon to comprise 53 target genes (Table 1). To determine the causal relationships between the binding of PurR and the changes in RNA transcript levels of genes in the PurR regulon, we integrated the information on the binding regions of PurR with transcriptomic analysis. Among 53 target genes in PurR regulon determined by ChIP-chip analyses, we determined 23 genes (43%) differentially expressed in response to the purR deletion and the addition of exogenous adenine with more than a 2-fold change and an FDR value <0.05 (P-value = 0.0056) from ANOVA analysis (Figure 1E and Supplementary Table S4). The genes directly repressed by PurR in response to the exogenous adenine (23 genes) include codB, codA, purT, xanP, yieG, pyrL, pyrB and pyrI encoding cytosine NCS1 transporter, cytosine deaminase, phosphoribosylglycinamide-formyltransferase, xanthine NCS2 transporter, adenine transporter, PyrL leader peptide, aspartate carbamoyltransferase and aspartate carbamoyltransferase regulatory subunit, respectively, as newly found members.
The remaining 57% of the genes had a direct association with PurR, lacking significant changes in RNA transcript levels. The cellular functions of most of the remaining genes were not clustered in purine and pyrimidine metabolic pathways, indicating that the changes in their transcript levels require additional regulatory signals such as transcription factors. Surprisingly, none of the genes were directly activated by PurR. On the contrary, PurR completely represses target genes involved in the IMP biosynthetic pathway. This suggests that most of the repression is a direct interaction, but the transcriptional activation is indirect (Supplementary Tables S1, S3 and S4).

In general, the PurR-binding sites are located in the promoter region between position −35 and −10 promoter elements, indicating that the binding of PurR regulates transcription initiation (2). In the case of purB and purR, PurR binds to the open reading frame so that it blocks transcription elongation (34). Therefore, the binding position of PurR is of great interest in order to understand its regulatory mechanism. We calculated the distance between PurR-binding motifs and the transcription start sites (TSSs) based upon the TSSs recently published (11). Of 32 promoter regions directly regulated by PurR, 14 regions (44%) include the PurR-binding motif between −10 and −35 promoter elements.

**Metabolic pathways directly regulated by PurR–Hx complex**

Purine nucleotide metabolism plays a critical role in various cellular activities. To identify the metabolic pathways regulated by PurR–Hx complex, the members of PurR regulon were functionally classified and further mapped to the *E. coli* metabolic pathways (Figure 2). The genes with direct PurR association lacking changes in transcript levels were classified into other cellular functions. However, the genes directly repressed by PurR in response to the exogenous adenine clustered mainly into purine and pyrimidine metabolic pathways. First, PurR directly autoregulates itself and generates PurR–Hx complex in the presence of Hx or adenine (Figure 2A). Second, PurR completely regulates purine transport, biosynthesis, salvage and interconversion pathways (Figure 2B). Interestingly, PurR directly regulates serine transport and metabolic pathways to produce N10-formyltetrahydrofolate (N10-FTHF), which is an intermediate for the IMP biosynthetic pathway. In addition, we found that PurR directly represses xanthine (*xanP*), purine nucleoside (*tsx*) and adenine (*yieG*) transporters. Although the transporter for Hx is currently unknown, xanthine transporter is unable to transport Hx (35). Lastly, PurR downregulates the genes in pyrimidine biosynthetic and transport pathways (Figure 2C). Most of the genes having direct PurR association with differential gene expression were enriched into purine and pyrimidine metabolic pathways. Interestingly, none of genes involved with purine utilization, such as *apt*, *deoD* and *add*, are directly regulated by PurR.

**DISCUSSION**

We determined the PurR regulon in *E. coli* in response to the exogenous adenine stimuli by integrating genome-scale location analysis and gene expression profiles. The genome-wide map of PurR-binding sites presented here not only confirms previously characterized binding sites (15 regions) but also expands the number of known binding sites (35 regions) to a genome-wide assessment; similar to what we previously reported for Lrp- and Fis-binding sites (10,32). From the genome-wide

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**Figure 2.** Metabolic pathways directly regulated by PurR and regulatory motif. (A) Formation of PurR–hypoxanthine complex. (B, C) The purine and pyrimidine biosynthesis pathways. The genes directly regulated by PurR are depicted by bold characters. Red arrows show the PurR-mediated repression. Broken arrows indicate the transporters. (D) Schematic diagram for the regulatory motif reconstruction in feedback loop.
mapping results, we were also able to show that: (i) a total of 35 PurR-binding regions were identified, all of which were located within noncoding regions, showing the strong binding preference of PurR-binding to the promoter and promoter-like regions; (ii) only 37% of binding sites (13 of 35) overlapped under the conditions in the absence and presence of exogenous adenine, indicating that PurR bindings to the E. coli genome are dramatically sensitive to the addition of exogenous adenine (or hypoxanthine); (iii) the integration of these results with mRNA transcript level information indicates that the functional assignment of the regulated genes is strongly enriched in the purine and pyrimidine metabolism-related functions. In addition, most of the genes were thoroughly repressed by PurR. Interestingly, the other genes directly bound by PurR lacking differential expression in response to the purR deletion or the exogenous adenine were functionally diverse; and (iv) the PurR-binding motifs were observed at the regions of −10 and −35 promoter elements, indicating PurR regulates transcription initiation.

We discovered PurR-binding regions from the promoter regions of codBA, purT, xanP, yieG and pyrLB with the differential gene expression. First, PurR directly regulates the de novo biosynthesis of pyrimidine. Among the genes in the biosynthetic pathway, codB and codA encode a cytosine transporter belonging to the NCS1 family of purine and pyrimidine transporters and a cytosine deaminase metabolizing cytosine to uracil and ammonia, respectively. In addition, PurR directly regulates pyrB and pyrL, encoding catalytic and regulatory subunits of aspartate transcarbamylase (ATCase), respectively, catalyzing the first reaction of the de novo biosynthesis of pyrimidine nucleotides. Considering that PurR represses carA, carB, pyrC and pyrD, the very early steps of de novo pyrimidine biosynthesis and transport are tightly regulated by PurR in response to exogenous adenine. Interestingly, RutR, the uracil responsive transcription factor, binds to the promoter region of carAB (36). Although Shimada and co-workers demonstrated that the RutR binding site plays little or no role in the regulation of transcription, it may have an additional regulatory role along with other proteins. In the carAB promoter, at least five regulatory proteins (IHF, PepA, PurR, RutR and ArgR) are involved in the purine, pyrimidine and arginine-specific control of the promoter activity (28). The complexity of the multicomponent regulatory mechanisms modulating carAB transcription shows the need for a cellular balance between the synthesis of pyrimidine and purine residues. Second, PurR directly regulates the transport of purine nucleotides. We observed PurR-binding peaks at the upstream regions of xanP, mdtL and yieG with differential gene expression in response to the exogenous adenine. Interestingly, PurR-binding peaks were observed for xanP and mdtL in the absence of exogenous adenine but not for yieG, suggesting that the yieG encodes a high-affinity transport system for adenine, which is dispensable in the presence of excess substrate. It has been suggested that another adenine transport system close to the genomic position of yieG operates at low affinity and is not energy dependent (30). However, it has not been discovered which gene has the adenine transport function with low affinity. Here, we found that mdtL encoding drug MFS transporter is directly repressed by PurR and located close to yieG (4.5 kb), indicating that mdtL might be responsible for the low-affinity adenine transport. Thus, the purine transport system of the PurR regulon can be composed of two high-affinity transporters (xanP and yieG) and one low-affinity transporter (mdtL).

Transcriptional regulatory systems often regulate the formation rates and the concentration of small molecules by feedback loops that regulate the transport, biosynthesis and metabolic enzymes (11,37). Since adenine can be utilized by apt, encoding adenine phosphoribosyltransferase, we were able to connect transport, biosynthesis and metabolic feedback loop pairs (Figure 2D). In the left loop, PurR–Hx complex represses the transcription of the transport proteins (T) for purine (xanP and yieG) and pyrimidine (codDB), and biosynthetic proteins (B) for IMP (purEK, purB, purT, purF, purC, purMN, purL and purHD) and UMP (carAB, codA, pyrBI and pyrCD), reducing the influx of the purine or pyrimidine molecules (P_in) from the media (P_out) and precursors (P_pres). In the right loop, metabolic enzyme (U) responsible for converting P_in into metabolites (M) is not directly regulated by PurR–Hx complex; however, its transcript level is reduced by the exogenous adenine. Thus, the logical structure of the connected feedback loop (CFL) motif described by a notation that uses three signs indicating repression (R) or activation (A) for each of T, B, and U can be R-R-R. In the previous studies (32,37), the B component (i.e. biosynthesis) was not included in the logical structures. The R-R-R motif demonstrates that the influx and efflux are repressed for flow homeostasis (37), which means that the exogenous adenine cannot be utilized as nutrient molecules. In the case of nutrient molecules and homeostasis, the logical structures of CFL would have been A-A/R-A and A-A/R-A, respectively (37). Since the R-R-R motif is uncommon for the regulation of small molecules in living cells, transcriptional regulatory networks for maintaining the levels of the purine and pyrimidine molecules may be more complex than previously thought.

Previously, the PurR was classified into the group of general TFs based on the functional diversity of the genes in its regulon (6). Compared to the other general TFs in E. coli such as Fur (38), Crp (39) and Lrp (32), the functions and size of the PurR regulon are limited. However, cellular functions of the genes are highly enriched into the purine and pyrimidine transport and biosynthesis, indicating that the direct effect of PurR on the E. coli metabolism is local, but via the balance of cellular purine content, it plays a critical role in metabolism. Now we may need to select a new list of global transcription factors in E. coli.

**ACCESSION NUMBER**

All raw data files have been deposited to Gene Expression Omnibus through accession numbers GSE26588 and GSE26589.
SUPPLEMENTARY DATA
Supplementary Data are available at NAR Online.

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