A Critical Role for the Putative NCS2 Nucleobase Permease YjcD in the Sensitivity of *Escherichia coli* to Cytotoxic and Mutagenic Purine Analogs

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**ABSTRACT** The base analogs 6-N-hydroxylaminopurine (HAP) and 2-amino-HAP (AHAP) are potent mutagens in bacteria and eukaryotic organisms. Previously, we demonstrated that a defect in the *Escherichia coli* *ycbX* gene, encoding a molybdenum cofactor-dependent oxidoreductase, dramatically enhances sensitivity to the toxic and mutagenic action of these agents. In the present study, we describe the discovery and properties of a novel suppressor locus, *yjcD*, that strongly reduces the HAP sensitivity of the *ycbX* strain. Suppressor effects are also observed for other purine analogs, like AHAP, 6-mercaptopurine, 6-thioguanine, and 2-amino-purine. In contrast, the *yjcD* defect did not affect the sensitivity to the pyrimidine analog 5-fluorouracil. Homology searches have predicted that *yjcD* encodes a putative permease of the NCS2 family of nucleobase transporters. We further investigated the effects of inactivation of all other members of the NCS2 family, XanQ, XanP, PurP, UacT, UraA, RutG, YgfQ, YicO, and YbbY, and of the NCS1 family nucleobase permeases CodB and YbbW. None of these other defects significantly affected sensitivity to either HAP or AHAP. The combined data strongly suggest that *YjcD* is the primary importer for modified purine bases. We also present data showing that this protein may, in fact, also be a principal permease involved in transport of the normal purines guanine, hypoxanthine, and/or xanthine.

**IMPORTANCE** Nucleotide metabolism is a critical aspect of the overall metabolism of the cell, as it is central to the core processes of RNA and DNA synthesis. At the same time, nucleotide metabolism can be subverted by analogs of the normal DNA or RNA bases, leading to highly toxic and mutagenic effects. Thus, understanding how cells process both normal and modified bases is of fundamental importance. This work describes a novel suppressor of the toxicity of certain modified purine bases in the bacterium *Escherichia coli*. This suppressor encodes a putative high-affinity nucleobase transporter that mediates the import of the modified purine bases. It is also a likely candidate for the long-sought high-affinity importer for the normal purines, like guanine and hypoxanthine.
N-hydroxylated agents, including the mechanisms of entry into the cell, we found that a transposon insertion in the yjcD locus strongly suppressed the HAP sensitivity of a ycbX mutant. YjcD encodes a hypothetical protein belonging to the nucleobase-transporter-2 (NCS2) family of perases that are involved in high-affinity transport of nucleobases (see http://www.tcdb.org). As shown in Fig. 1, the E. coli genome contains 10 related paralogous members of the NCS2 family: the uracil perase UraA (12), the xanthine-specific transporters XanQ and XanP (13), the putative adenine perase PurP (14, 15), the uric acid transporter UacT (16), the putative uracil/thymine perase RutG (17), and four additional hypothetical transporters, YjcD, YbbY, YicO, and YgfQ. E. coli also contains two members of the NCS1 family of perases, among which CodB was characterized as a cytosine-specific transporter (18) and YbbW, which is particularly sensitive to 5-flourouracil (FU) (20). The results in Fig. 2 show that the yjcD mutation also suppressed this effect. In contrast, no effect on the cytoxicity of 5-flourouracil was seen for the yjcD deletion (Fig. 2). As the yjcD gene encodes a hypothetical protein belonging to the NCS2 family of nucleobase transporters and YbbW, may also be involved in base analog uptake (see the introduction).

In the present study, we describe the properties of the yjcD strain with regard to its resistance to various base analogs. We also construct a set of strains carrying defined deletions of each of the members of the NCS2 and NCS1 families for an examination of any influence that these mutations may have on base-analog sensitivity. Our results suggest a pivotal role of YicO in the uptake of HAP and related purine base analogs in E. coli. These results are discussed with regard to the possible physiological function of the YjcD transporters.

RESULTS AND DISCUSSION

A yjcD defect suppresses the cytotoxic effect of purine base analogs. A mutant containing a defect in yjcD was originally isolated in a genome-wide search using random transposon insertion mutagenesis for mutations that could suppress the HAP hypersensitivity of an ycbX mutant defective in base analog detoxification (E. I. Stepchenkova, S. G. Kozmin, and R. M. Schaaper, unpublished data). Here, we demonstrate that a ΔycbX strain carrying a defined deletion of the yjcD gene displays a strong reduction in sensitivity to the toxic effects of HAP or AHAP: for HAP, the zone of inhibition decreased from 39 mm to 18 mm, while for AHAP, the inhibition zone decreased from 36 mm to 0 (see Fig. 2). The ΔyjcD mutant also suppressed the sensitivity of a wild-type strain toward the toxic action of the purine analogs 6-mercapto purine (MP) (from a clear 50-mm zone to a 25- to 30-mm diffuse zone of inhibition) and 6-thioguanine (TG) (50 versus 0 mm) (Fig. 2). The effect of ΔyjcD on the sensitivity to the purine analog 2-aminopurine (AP) was tested in a dam strain background, which is particularly sensitive to this agent (20). The results in Fig. 2 show that the ΔyicO mutation also suppressed this effect. In contrast, no effect on the cytoxicity of AP was seen for the yicO deletion (Fig. 2). As the yicO gene encodes a hypothetical protein belonging to the NCS2 family of nucleobase transporters (1), the suppressor effect of the yjcD deletion is most plausibly ascribed to defective transport of the tested purine analogs into the cell.

Other members of the NCS2 and NCS1 families do not affect HAP and AHAP sensitivity. As shown in Fig. 1, E. coli possesses 10 members of the NCS2 protein family, including YjcD. Four of these proteins have been characterized as purine-specific transporters (XanQ, XanP, PurP, and UacT), while two are implicated in the transport of pyrimidines (UraA and RutG) (see the introduction). Four other members of NCS2 family, YjcD, YgfQ, YicO, and YbbY, are hypothetical proteins. In addition, two members of the NCS1 family of nucleobase transporters, CodB and YbbW, may also be involved in base analog uptake (see the introduction).

We investigated the role of each of these genes in sensitization to HAP and AHAP by analyzing the sensitivity of the ycbX strain carrying the corresponding gene deletions (see Table 1). As shown in Fig. 3, none of the deletions tested significantly affected sensitivity to HAP (36- to 39-mm inhibition zones for all mutants except ΔyjcD) and AHAP (36- to 38-mm inhibition zones for all mutants except ΔyjcD). In the ΔycbX ΔyicO mutant, a weak background growth was noted within the HAP-induced inhibition zone (Fig. 3A). To more carefully investigate the possible operation of transporters other than YjcD, we further combined defects in all NCS2 and NCS1 family proteins with the ΔyjcD deletion. As shown in Fig. 4, none of the deletions tested were capable of eliminating the residual HAP sensitivity of the yjcD mutant; again, a weak yicO-associated suppression of the HAP sensitivity was observed.

While the results in Fig. 3 and 4 regarding the role of YicO may be taken to suggest that this gene has a limited involvement in HAP uptake, an alternative explanation, which we favor, is that the reduced HAP sensitivity of the ΔycbX::kan strain that we used (Table 1) is due to activation of the adjacent yicP (ade) gene, encoding an adenine deaminase (21, 22) which is capable of HAP detoxification by conversion of HAP to hypoxanthine (11). See footnote b of Table 1 for details. This explanation is further consistent with the lack of effect of the ΔyicO mutation on sensitivity to AHAP (Fig. 3B) or MP and TG (see Fig. S1 in the supplemental material). Thus, our results demonstrate a major role of, specifically, YjcD in the uptake of a series of modified purines and, fur-
thermore, that none of the other NCS2 and NCS1 family members play any significant role in the uptake of these analogs.

The effect of yjcD on uptake of natural purines. In addition to playing a major role in the uptake of modified purines, YjcD might also play a role in the uptake of normal purines, like adenine, guanine, hypoxanthine, and xanthine. To test this possibility, we first used a purC strain, defective in de novo purine biosynthesis, and assayed its growth with either adenine or hypoxanthine as the purine source. The results in Fig. 5A and B show that growth on adenine is entirely unaffected by the yjcD mutation, consistent with the existence of the high-affinity PurP adenine transporter (14, 15), but growth on hypoxanthine is significantly delayed. Second, we tested the growth of a guaB strain, which lacks inosine monophosphate (IMP) dehydrogenase activity and, hence, requires the presence of either xanthine or guanine for growth (23). The results in Fig. 5C show strongly impaired growth of the yjcD derivative when guanine serves as the purine source, while a modest growth defect was seen in the presence of xanthine (Fig. 5D).

Possible physiological role of YjcD. The membership of YjcD in the NCS2 family clearly classifies the protein as a nucleobase transporter. No direct measurements of transport activities by this protein have been reported at this time, but several observations support this particular role for YjcD. Using C-terminal tagging with reporter proteins, YjcD was shown to be localized in the bacterial inner membrane (24). It was also shown that the yjcD promoter contains a PurR purine repressor binding motif, which is present in various genes involved in purine and pyrimidine metabolism, including several transporters (25). In agreement with the latter, PurR-dependent repression of yjcD expression by exogenous adenine was reported in an independent study (26). We, likewise, observed a 4-fold down-regulation of yjcD gene expression in a microarray experiment upon the addition of 50 μg/ml of HAP to minimal growth medium (S. G. Kozmin and R. M. Schaaper, unpublished data). This is an important result because, in wild-type strains, HAP is readily converted to adenine by the YcbX/YiiM-mediated reduction reaction (in this manner, HAP can serve as a ready purine source for purine-requiring mutants), but this reaction and the subsequent repression of the purR regulon require HAP entry into the cell.

What may be the natural substrate(s) for YjcD permease? The protein is clearly important for the uptake of the tested purine analogs HAP, AHAP, MP, TG, and AP, as illustrated here. As one other example, in molybdenum-cofactor-deficient strains, HAP is already highly toxic at very low nanomolar concentrations in the growth medium, while mutagenesis by HAP, reflecting incorporation into the DNA, can be observed at concentrations as low as 0.05 nM (27). Indeed, the incorporation of HAP into both the RNA and DNA of the cell is rapid and observable within minutes (Z. Nguyen and R. M. Schaaper, unpublished data), consistent with efficient uptake of HAP from the medium. Thus, YjcD enables efficient scavenging of very low concentrations of purine analogs from the medium. It might thus be argued that YjcD represents a broad-specificity transporter for a variety of modified purines, including both adenine analogs like HAP and 6-mercaptopurine and guanine analogs like AHAP, 6-thioguanine, and 2-aminopurine.

However, our other results, as described for the experiments whose results are shown in Fig. 5, also suggest that YjcD may represent a transporter for the natural purines guanine, hypoxan-

### FIG 2
Sensitivities of yjcD+ and ΔyjcD strains to the toxic effect of various base analogs. Cell suspensions were transferred using a multiprong replicator to VB plates, and 100 μg of HAP, 50 μg of AHAP or 200 μg of MP, TG, AP, or FU (in DMSO solutions) was spotted onto the center of each plate. The plates were incubated overnight at 37°C and inspected the next day for zones of inhibition. For HAP and AHAP sensitivity tests, NR16262 (ΔycbX) and its ΔyjcD::tet derivative were used. For MP, TG, and FU sensitivity tests, NR10836 (wild type [wt]) and its ΔyjcD::tet derivative were used. For the AP sensitivity test, we used strain NR15719 (dam) and its ΔyjcD::kan derivative.
When using the purP::kan mutation, putatively places the yjcD gene, which is located directly adjacent to the deletion endpoints, whereas upper case letters indicate the sequences of pKD13 or Tn10. The orientation of antibiotic resistance markers in all deletion-insertion mutants is clockwise.

| Deletion | Affected gene(s) | PCR primer pairs used to generate corresponding deletion |
|----------|------------------|----------------------------------------------------------|
| ΔyjcD::tet | yjcD | 5’t ttg ttc tat gac gcc atc agc ggg tac cgg tgc acc cga cta AGA GGG GGA CTA TTA TAT TTC G 3’ |
| ΔyicO::kan | yicO | 5’ aac ggg caa ctc ttc aga ata cta gta aag att ata AAT CTC GAC ATC TGG GTG ACC G 3’ |
| ΔpurP::kan | purP | 5’ gcc cgc ctc cgc atc agc ttc atg ata gct tgc cgc ctc gga gaa gaa taa at tct ggc gaa GGA TGG GTG ACC GAG CTG CTG CCT CG 3’ |
| Δ(xanQ-guaT)::kan | xanQ guaD ygfF Q ygfS ygfT uacT | 5’ gca gta gga gat taa ggc cgc gac gcc atc acc acc ctc ggt att gga gaa gaa AAT TCC CGG GGA TCC GTC GAC C 3’ |
| ΔxanP::kan | xanP | 5’ acg cgt ctg cca ggc tgc cgg ccc ccc aac ttc gtg gga att tat ttt ttc ggg AGT AGG CTG GAG CTG CTG CG 3’ |
| ΔrutG::kan | rutG | 5’ gac act ggc ggt gta atg gcc ggc gga gaa gaa att ctc ttc ttc ttc ggg GGA TCC GTC GAG CTG CG 3’ |
| ΔuraA::kan | uraA | 5’ tcc ctc ctc ctc ctc ctc ctc ctt ctc ctt ccc ctc ggg AGT AGG CTG GAG CTG CTG CG 3’ |
| Δ(ybbW-ybbY)::kan | ybbW ybbY | 5’ tga gta aag cgc cta taa cac atc ata cag agg ata atca tca gta gca gca gca gga GGA TGG GTG ACC GAG CTG CTG CG 3’ |
| ΔcodB::kan | codB | 5’ aac aac cca gca aac ggg aat ttc gtc tgt gaa gaa gaa cgg cag cgg GGA TGG GTG ACC GAG CTG CTG CG 3’ |

| TABLE 1 | Gene deletions generated in this study |
|----------|--------------------------------------|
| Deletion | Affected gene(s) |
| ΔyjcD::tet | yjcD |
| ΔyicO::kan | yicO |
| ΔpurP::kan | purP |
| Δ(xanQ-guaT)::kan | xanQ guaD ygfF Q ygfS ygfT uacT |
| ΔxanP::kan | xanP |
| ΔrutG::kan | rutG |
| ΔuraA::kan | uraA |
| Δ(ybbW-ybbY)::kan | ybbW ybbY |
| ΔcodB::kan | codB |

| a Lowercase letters indicate sequences corresponding to the deletion endpoints, whereas upper case letters indicate the sequences of pKD13 or Tn10. The orientation of antibiotic similarity between YjcD and YgfQ (16), the latter may be thought to be a candidate for such a system. However, our experiments with a ygfQ deletion did not reveal increased resistance to HAP or AHAP, either singly or in combination with ΔyjcD (Fig. 3 and 4; Fig. S1), or to the analogs MP or TG (Fig. S1). With regard to growth supported by xanthine, XanQ and XanP have recently been described as xanthine-specific transporters (13), and their action may account for the limited effect of the ΔyjcD mutation on xanthine utilization (Fig. 5D). Nevertheless, our data imply some positive role of YjcD in xanthine utilization.

It is of interest to note that the proteins implicated in the transport of adenine (PurP) or guanine and hypoxanthine (YjcD) belong to a separate cluster of orthologous groups (COG2252 at the NCBI database), represented by the lower four proteins in Fig. 1. This group is distinct from that formed by the upper six proteins (COG2233), which represent permeases involved in the transport of 2-oxopurines (xanthine and uric acid) or pyrimidines. The COG2252 proteins of E. coli display high sequence conservation in certain consensus sites of the TM1, TM3, TM8, TM9, TM10, and TM14 transmembrane segments, which contain amino acids that are functionally important but differ from those of the COG2233 members (16, 29). Thus, the distinct conservation pattern of
COG2252 genes at these sites may reflect their specificity toward 2-nonoxidized purines (adenine, guanine, and hypoxanthine).

MATERIALS AND METHODS

Media and chemicals. Bacteria were cultivated in LB broth (30) or minimal Vogel-Bonner medium (VB) (31) containing 0.2% glucose as the carbon source and supplemented with 1 g/ml of thiamine. Solid medium contained 1.5% agar. For selection of antibiotic-resistant clones, media were supplemented with 35 g/ml of kanamycin or 15 g/ml of tetracycline. HAP was purchased from Midwest Research Institute (Kansas City, MO). AHAP was obtained from Ilya Kuchuk (Indiana University). 2-Aminopurine was purchased from Monomer-Polymer and Dajac Laboratories, Inc. (Trevose, United States). All other chemicals were from Sigma-Aldrich.

Bacterial strains. All gene deletions (Table 1) were initially generated in E. coli strain BW25113 (lacIq rrlB14 ΔlacZΔM15 hsdR514 ΔaraBAD165 ΔrhaBAD14774) by the PCR-based gene-replacement method of Datsenko and Wanner (32), using either the Kan module of plasmid pKD13 (32) or the tetA tetR tetracycline resistance (Tetr) module of transposon Tn10 as a template. The resulting deletion-insertions were then transferred into E. coli strain NR10836 [ara thi Δ(pro-lac) F′ CC106] (27) or its ΔycbX derivative NR16262 (11) by P1 transduction using P1virA. To combine the yjcD deficiency with mutations in the genes encoding other NCS2 and NCS1 family proteins, the corresponding gene deletion-insertions were further transferred into the NR16262-ΔycbX derivative by P1 transduction.

To generate the dam yjcD double mutant, the ΔyjcD758::kan allele from Keio collection strain JW4025 (33) (National BioResource Project [NIG, Japan]: E. coli) was introduced by P1 transduction into strain NR15719, an NR10836 derivative carrying a dam::mini-Tn10cam insertion (our strain collection). To generate the purC yjcD and guaB yjcD double mutants, the ΔyjcD758::kan allele from Keio collection strain JW4025 was introduced by P1 transduction into strain NR15791, an NR10836 derivative carrying the purC::mini-Tn10 insertion (34), or into strain NR17097, an NR10836 derivative carrying a precise in-frame ΔguaB deletion (i.e., not affecting guaA expression) created by the method of Datsenko and Wanner (32).

Spot test for HAP sensitivity. Saturated E. coli cultures grown in LB were diluted 30-fold in 0.9% NaCl and transferred to VB plates using a multiprong replicator device (approximately 0.1 ml total per plate). After the spots had dried, an appropriate volume of a 10- to 40-mg/ml solution of HAP, AHAP, MP, TG, AP, or FU in dimethyl sulfoxide (DMSO) or DMSO only was spotted onto the center of the plate. The plates were incubated overnight at 37°C and inspected the next day for zones of inhibition.

FIG 3 HAP-induced killing (A) and AHAP-induced killing (B) of E. coli strains lacking various NCS2 and NCS1 family proteins. The strains used were NR16262 (ΔycbX) and its derivatives carrying the indicated deletions. Note that the Δ(xanQ-uacT) deletion also includes the ygfQ gene (Table 1). Spot tests were performed as described in the legend to Fig. 2 and in Materials and Methods. An amount of 100 μg of HAP or 50 μg of AHAP was applied to the center of each plate.
Growth kinetics of *E. coli* cultures in presence of purine bases. Derivatives of strain NR10836 containing the purC, purC yjcD, guaB, or guaB yjcD defects (see above) were grown in liquid LB medium at 37°C to saturation. For each culture, 100-μl aliquots were collected by centrifugation, the supernatants removed, and the pellets resuspended in 1 ml of 0.9% NaCl. Five microliters of these suspensions were inoculated into 250 μl of VB medium supplemented with one of the purine bases tested (adenine, hypoxanthine, guanine, or xanthine) at a 0.1 mM concentration.

**FIG 4** HAP sensitivities of *E. coli* strains carrying a yjcD defect combined with defects in other NCS2 and NCS1 family proteins. Note that the Δ(xanQ-uacT) deletion also includes the ygfQ gene (Table 1). The experiments were performed in the NR16262 (ΔycbX) genetic background. Spot tests were performed as described in the legend to Fig. 2 and in Materials and Methods. An amount of 200 μg of HAP was applied to each plate.

**FIG 5** Effect of the yjcD defect on growth of the purC or guaB purine auxotrophs in the presence of various purine sources. (A and B) Growth of a purC purine auxotroph in the presence of adenine (A) or hypoxanthine (B). (C and D) Growth of a guaB purine auxotroph in the presence of guanine (C) or xanthine (D). For each time point, the average $A_{600}$ value obtained for 12 independent cultures is presented, with the standard deviation indicated by the error bar. The background $A_{600}$ absorbance, equal to 0.07, was not subtracted from the measurements. VB indicates the Vogel-Bonner minimal medium used in the experiment along with the noted purine supplement. See Materials and Methods for more details.
in a 96-well microplate. Growth curves were recorded at 37°C without shaking, with $A_{600}$ readings taken every 1 h using a Tecan microplate reader (Tecan Group Ltd., Männedorf, Switzerland).

**SUPPLEMENTAL MATERIAL**

Supplemental material for this article may be found at http://mbio.asm.org/lookup/suppl/doi:10.1128/mBio.00661-13/-/DCSupplemental.

**ACKNOWLEDGMENTS**

We thank Kelly Daughtry and Mark Itsko of the NIEHS for their helpful comments on the manuscript.

This work was supported by project ES050170 of the Intramural Research Program of the National Institute of Environmental Health Sciences (NIEHS).

**REFERENCES**

1. Barrett JC. 1981. Induction of gene expression and in cell transformation of mammalian cells by modified purines: 2-aminopurine and 6-N-hydroxylaminopurine. Proc. Natl. Acad. Sci. U. S. A. 78:5685–5689.
2. Pavlov YI, Noskov VN, Lange EK, Moiseeva EV, Pshenichnov MR, Khromov-Borisov NN. 1991. The genetic activity of N'-hydrox adenine and 2-amino-N6-hydroxy adenine in *Escherichia coli*, *Salmonella typhimurium* and *Saccharomyces cerevisiae*. Mutat. Res. 253:33–46.
3. Kozmin SG, Schaaper RM, Scherbakova PV, Kulikov VN, Noskov VN, Guetsova ML, Alenin VV, Pavlov YI. 1998. Multiple antimutagenesis mechanisms affect mutagenic activity and specificity of the base analog 6-N-hydroxylaminopurine in bacteria and yeast. Mutat. Res. 402:41–50.
4. Khromov-Borisov NN. 1997. Naming the mutagenic nucleic acid base analogs: the Galatea syndrome. Mutat. Res. 379:233–240.
5. Clement B, Kunze T. 1990. Hepatic microsomal N-hydroxylation of adenine to 6-N-hydroxylaminopurine. Biochem. Pharmacol. 39:925–933.
6. Lieberman I. 1956. Enzymatic synthesis of adenine-5'-phosphate from inosine-5'-phosphate. J. Biol. Chem. 223:327–339.
7. Simandan T, Sun J, Dix TA. 1998. Oxidation of DNA bases, deoxyribo nucleosides and homopolymers by peroxyl radicals. Biochem. J. 335:233–240.
8. Stepchenkova EI, Kozmin SG, Alenin VV, Pavlov YI. 2005. Genome-wide screening for genes whose deletions confer sensitivity to mutagenic purine base analogs in yeast. BMC Genet. 6:31. doi:10.1186/1471-2156-6-S1-S31.
9. Stepchenkova EI, Kozmin SG, Alenin VV, Pavlov YI. 2009. Genetic control of metabolism of mutagenic purine base analogs 6-hydroxylaminopurine and 2-amino-6-hydroxyadenine in yeast *Saccharomyces cerevisiae*. Genetika 45:471–477.
10. Burgis NE, Brucker JJ, Cunningham RP. 2003. Repair system for non-canonical purines in *Escherichia coli*. J. Bacteriol. 185:3101–3110.
11. Kozmin SG, Leroy P, Pavlov YI, Schaaper RM. 2008. YcbX and yilM, two novel determinants for resistance of *Escherichia coli* to N-hydroxylated base analogues. Mol. Microbiol. 68:51–65.
12. Andersen PS, Frees D, Fast R, Mygind B. 1995. Uracil uptake in *Escherichia coli* K-12: isolation of uraA mutants and cloning of the gene. J. Bacteriol. 177:2008–2013.
13. Karatza P, Frillingos S. 2005. Cloning and functional characterization of two bacterial members of the NAT/NC2 family in *Escherichia coli*. Mol. Membr. Biol. 22:251–261.
14. Burton K. 1983. Transport of nucleic acid bases into *Escherichia coli*. J. Gen. Microbiol. 129:3505–3513.
15. Burton K. 1994. Adenine transport in *Escherichia coli*. Proc. Biol. Sci. 255:153–157.
16. Papakostas K, Frillingos S. 2012. Substrate selectivity of YgU, a uric acid transporter from *Escherichia coli*. J. Biol. Chem. 287:5684–5695.
17. Kim KS, Pelton JG, Inwood WB, Andersen U, Kustu S, Wemmer DE. 2010. The Rut pathway for pyrimidine degradation: novel chemistry and toxicity problems. J. Bacteriol. 192:4089–4102.
18. Danielsen S, Kilstrup M, Barilla K, Joehimsen B, Neuhard J. 1992. Characterization of the *Escherichia coli* codBA operon encoding cytosine permease and cytosine deaminase. Mol. Microbiol. 6:1335–1344.
19. Cusa E, Obradors N, Baldomà L, Badia J, Aguilar J. 1999. Genetic analysis of a chromosomal region containing genes required for assimilation of allantoin nitrogen and linked glyoxylate metabolism in *Escherichia coli*. J. Bacteriol. 181:7479–7484.
20. Glickman B, van den Elen P, Radman M. 1978. Induced mutagenesis in dam” mutants of *Escherichia coli*: a role for 6-methyladenine residues in mutation avoidance. Mol. Gen. Genet. 163:307–312.
21. Zalkin H, Nygaard P. 1996. Biosynthesis of purine nucleotides, p 561–579. In Neidhardt FC (ed.), *Escherichia coli* and *Salmonella*: cellular and molecular biology. ASM Press, Washington, DC.
22. Matsui H, Shimaoka M, Kawasaki H, Takenaka Y, Kurahashi O. 2001. Adenine deaminase activity of the yciP gene product of *Escherichia coli*. Biosci. Biotechnol. Biochem. 65:1112–1118.
23. Lampden PR, Drabble WT. 1973. The gus operon of *Escherichia coli* K-12: evidence for polarity from gusA to gusB. J. Bacteriol. 115:992–1002.
24. Daley DO, Rapp M, Granseth E, Melén K, Drew D, von Heijne G. 2005. Global topology analysis of the *Escherichia coli* inner membrane proteome. Science 308:1321–1323.
25. Cho BK, Fedorowicz SA, Embree M, Park YS, Kim D, Palsson BØ. 2011. The PurR regulon in *Escherichia coli* K-12 MG1655. Nucleic Acids Res. 39:e456–e464.
26. Marbach D, Costello JC, Kuffner R, Vega NM, Prill RJ, Camacho DM, Allison KR, DREAM5 Consortium, Kellis M, Collins JJ, Stolovitzky G. 2012. Wisdom of crowds for robust gene network inference. Nat. Methods 9:796–804.
27. Kozmin SG, Pavlov YI, Dunn RL, Schaaper RM. 2000. Hypersensitivity of *Escherichia coli* Δ(uvrB::bio) mutants to 6-hydroxylaminopurine and other base analogs is due to a defect in molybdenum cofactor biosynthesis. J. Bacteriol. 182:3361–3367.
28. Roy-Burman S, Visser DW. 1975. Transport of purines and deoxyadenosine in *Escherichia coli*. J. Biol. Chem. 250:9270–9275.
29. Karena E, Frillingos S. 2011. The role of transmembrane segment TM3 in the xanthine permease XanQ of *Escherichia coli*. J. Biol. Chem. 286:39595–39605.
30. Miller JR. 1972. Experiments in molecular genetics. Cold Spring Harbor, New York, NY.
31. Vogel HJ, Bonner DM. 1956. Acetylornithinase of *Escherichia coli* partial purification and some properties. J. Biol. Chem. 218:97–106.
32. Datsenko KA, Wanner BL. 2000. One-step inactivation of chromosomal genes in *Escherichia coli* K-12 using PCR products. Proc. Natl. Acad. Sci. U. S. A. 97:6640–6645.
33. Baba T, Ara T, Hasegawa M, Takai Y, Okumura Y, Baba M, Datsenko KA, Tomita M, Wanner BL, Mori H. 2006. Construction of *Escherichia coli* K-12 in-frame, single-gene knockout mutants: the Keio collection. Mol. Syst. Biol. 2:2006.0008. doi:10.1038/msb4100050.
34. Singer M, Baker TA, Schnitzler G, Deichsel SM, Goel M, Dove W, Jaacks KJ, Grossman AD, Erickson JW, Gross CA. 1989. A collection of strains containing genetically linked alternating antibiotic resistance elements for genetic mapping of *Escherichia coli*. Microbiol. Rev. 53:1–2.