Defect of RNA pyrophosphohydrolase RppH enhances fermentative production of l-cysteine in Escherichia coli

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Fermentative production of l-cysteine has been established using Escherichia coli. In that procedure, thiosulfate is a beneficial sulfur source, whereas repressing sulfate utilization. We first found that thiosulfate decreased transcript levels of genes related to sulfur assimilation, particularly whose expression is controlled by the transcription factor CysB. Therefore, a novel approach, i.e. increment of expression of genes involved in sulfur-assimilation, was attempted for further improvement of l-cysteine overproduction. Disruption of the rppH gene significantly augmented transcript levels of the cysD, cysJ, cysM and yeeE genes (≥1.5-times) in medium containing sulfate as a sole sulfur source, probably because the rppH gene encodes mRNA pyrophosphohydrolase that triggers degradation of certain mRNAs. In addition, the ∆rppH strain appeared to preferentially uptake thiosulfate rather than sulfate, though thiosulfate dramatically reduced expression of the known sulfate/thiosulfate transporter complexes in both ∆rppH and wild-type cells. We also found that both YeeE and YeeD are required for the strain without the transporters to grow in the presence of thiosulfate as a sole sulfur source. Therefore, yeeE and yeeD are assigned as genes responsible for thiosulfate uptake (tsuA and tsuB, respectively). In final, we applied the ∆rppH strain to the fermentative production of l-cysteine. Disruption of the rppH gene enhanced l-cysteine biosynthesis, as a result, a strain producing approximately twice as much l-cysteine as the control strain was obtained.

Key Words: fermentative production; Sulfur Index; thiosulfate; yeeD; yeeE
Abbreviations: AmpR, ampicillin-resistant gene; CmR, chloramphenicol-resistant gene; KmR, kanamycin-resistant gene; KPM, potassium-phosphate-magnesium; mBBr, monobromobimane; TS, thiosulfate

Introduction

Triposphates of the 5′-end of bacterial mRNA functions to protect mRNA from RNases, such as RNase E and RNase J (Foley et al., 2015; Mackie, 2013; Richards et al., 2011). RppH (also called NudH, YgdP) in Escherichia coli belongs to the Nudix hydrolase family and possesses RNA pyrophosphohydrolase activity removing pyrophosphate from the 5′-end of mRNA. Therefore, RppH is a factor for modulation of mRNA level through the initiation of mRNA breakdown, whereas no activity of RNA polymerase nor RNase (Deana et al., 2008).

Disruption of the rppH gene augments hundreds of, but not all, mRNA in E. coli (Deana et al., 2008; Luciano et al., 2012), implicating substrate selectivity of RppH. The substrate specificity has been first investigated in Bacillus subtilis RppH, of which substrate mRNAs must have at least two unpaired nucleotides in its 5′ region and “G” at the second nucleotide from the 5′-end (Hsieh et al., 2013; Piton et al., 2013). The E. coli enzyme claims two or more unpaired nucleotides at the 5′-end of substrate mRNAs (Foley et al., 2015; Vasilyev and Serganov, 2015) whilst its sequence preference is still unclear. Unlike in the case

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of eukaryotes, the stem-loop structure of the 5′ region of mRNA is not usual in bacteria, implying that many of E. coli mRNAs possess unpaired sequences at the 5′-end. At this moment, therefore, it is difficult to predict substrates of the E. coli enzyme in silico.

In E. coli utilizing inorganic sulfur such as sulfate, the sulfur assimilation pathway producing L-cysteine has been well characterized (Guédon and Martin-Verstraete, 2006; Kawano et al., 2018; Kredich, 1992). Intracellular L-cysteine level is restricted since an excess amount of this amino acid causes the adverse effect on cell growth and viability (Datta, 1967; Park and Imlay, 2003). The molecular basis of regulation of the pathway is well-known as a feedback inhibition of L-serine O-acetyltransferase CysE by L-cysteine (Kredich and Tomkins, 1966). Recently, it was reported that a transcription factor DecR plays a key role in detoxification of L-cysteine through regulation of yhaOM expression (Shimada et al., 2016).

In addition, N-acetyl L-serine produced spontaneously from O-acetyl L-serine (OAS) activates a transcription factor CysB, resulting in promoting expression of genes encoding sulfur assimilation-related enzymes (Flavin and Slaughter, 1965; Ostrowski and Kredich, 1989, 1990), such as CysC, CysD, CysH, CysI, CysJ, CysK, and CysN. Hence, these mechanisms probably cooperate to maintain a balance between supplies of substrates for L-cysteine synthase A CysK: OAS as a carbon+nitrogen source and sulfide as a sulfur source.

An E. coli system for L-cysteine-overproduction has been established by construction of a strain ectopically expressing the feedback inhibition-insensitive form of CysE (Denk and Böck, 1987; Kai et al., 2006) and L-cysteine exporters, such as YdeD and TolC (Daßler et al., 2000; Wiriyathanawudhiwong et al., 2009). More recently, it was also demonstrated that disruption of the yciW gene encoding a putative oxidoreductase further facilitates L-cysteine production (Kawano et al., 2015a, b). In addition, thiosulfate-containing medium has a potential for

Fig. 1. Thiosulfate selectively reduces expression of sulfur-related genes.
A. and B. Microarray assay for transcript level was carried out in E. coli cells that were incubated for 10 min in the presence/absence of thiosulfate. A. Transcript level of each gene in M9+TS medium was described as a value relative to that of wild-type in M9 medium. Open bars, wild-type; closed bars, ΔrppH. B. Data of wild-type cells were scatter-plotted to visualize the effect of thiosulfate. Cells were incubated in the absence of thiosulfate (M9) or in the presence of thiosulfate (M9+TS). Sulfur assimilation-related genes under control of CysB, and not under control of CysB (red and blue dots, respectively); cysB (a green dot); rpoH (internal control, an orange dot). C. Reporter assay using the plux system was performed to examine CysB regulation of sbp and yeeED gene/operon. The chemiluminescence intensity at 40 min after transfer to a fresh M9 medium was determined as promoter activity. Promoter activity in ΔcysB cells was plotted as a relative value to that in wild-type cells (means ± SE, n = 3). The promoter of cysJIH operon was used for CysB-regulated promoter control. The promoters of cysG and cysM genes were used as control promoters not regulated by CysB. n.d. indicates not detected.
fermentative production of l-cysteine, since thiosulfate is directly utilized for l-cysteine biosynthesis through an energy-saving pathway: S-sulfo-l-cysteine production from thiosulfate and OAS by cysteine synthase B CysM, S-sulfo-l-cysteine reduction forming l-cysteine by certain disulfide reductases, such as Grx1 and NrdH (Nakatani et al., 2012; Sirko et al., 1987). Byproducts, sulfide and sulfite in the reactions are applied to l-cysteine production through the sulfate pathway (Nakatani et al., 2012). However, in the presence of thiosulfate, cells appeared not to be able to utilize sulfate as a sulfur source (Nakatani et al., 2012; van der Ploeg et al., 2001). In this study, we first demonstrated that thiosulfate significantly attenuates transcript levels of sulfur assimilation-related genes whose expression is regulated by CysB. Thus, we have focused on a trigger of mRNA degradation RppH, because disruption of the rppH gene increases mRNA levels of some sulfur metabolism-related proteins, such as l-cystine transporter, FltY, an inner membrane protein YeeE that contains the “Sulf_transp” module and a putative sulfur trans- ferase Yeed (Deana et al., 2008; Luciano et al., 2012). In the end, the ability of E. coli to produce l-cysteine in the presence of thiosulfate was successfully improved by disruption of the rppH gene.

Materials and Methods

Bacterial strains and culture media. The E. coli strains used in this study are listed in Table S1. Gene disruption was performed as previously described (Baba et al., 2006; Datsenko and Wanner, 2000). “M9 medium” in this study means M9 medium modified in so far as 1 mM MgSO4 is changed to 1 mM MgCl2 and Na2SO4 is supplied for the sulfur source. M9+TS medium contains 1 mM thiosulfate as an additional sulfur source. SM1 medium was used for l-cysteine fermentative production (Wiriyathanawudhiwong et al., 2009).

Microarray analysis. E. coli strains, wild-type and ∆rppH were cultured at 30°C in M9 medium until reaching OD660 = 0.8. Cells were divided into two media, one into M9 and another into M9+TS, after harvesting and washing three-times with fresh M9 medium. After further incubation for 0, 5 and 10 min, cells were harvested and quickly frozen in liquid nitrogen. The following steps for analysis were performed by Takara Bio Inc., according to the protocol of Agilent Expression Array Analysis.

Promoter assay. Promoter assay was carried out using the pLux bioluminescence system (Burton et al., 2010; Tobe et al., 2014). About 300 bp or 600 bp of 5' flanking sequence of the gene was amplified (Table S2) and fused to the lux operon of Photorhabdus luminescens in the pLux plasmid using Xhol/RaelHI sites (Table S3). Transformed cells were cultured at 30°C in M9 medium until reaching OD660 = 0.3 and were dispensed into 3 wells in a 96-well plate. During further incubation at 30°C in M9 or M9+TS with shaking at 120 rpm, luminescence from cells was monitored for 60 min or longer with a luminometer (Mithras LB940, Berthold Technologies GmbH & Co.). A luminescence intensity at 40 min was used for promoter activity, since cells need approximately 35 min to accli- matize to fresh medium conditions (Burton et al., 2010).

Sulfate uptake assay. Uptake of SO42− into E. coli cells was determined as previously described (Ohtsu et al., 2010), except for using [35S]-labeled Na2SO42−.

“Sulfur Index” analysis. Intracellular S-containing metabolites were determined by the “Sulfur Index” analysis (Kawano et al., 2015b; Morikawa et al., 2012). Briefly, an E. coli cell pellet was resuspended in methanol containing 1 mM monobromobimane (mBBr) (M1378, Invitrogen) and 5 μM D-camphor-10-sulfonic acid (037-01032, Fujifilm Wako Pure Chemical Co.) as an internal control. Bimane-labeled compounds in extract were separated with liquid chromatography and identified by mass-spectrometer (LC-MS/MS) (LCMS-8030, Shimadzu Co.). Quantitative data was described as a peak area of a target compound relative to that of the D-camphor-10-sulfonic acid.

Challenge of Cysteine overproduction. The BW25113 E. coli strain transformed with the pDES plasmid (Table S3) was used as a control for l-cysteine production (Wiriyathanawudhiwong et al., 2009). Cells were inoculated into 1.1 L of SM1 medium and cultivated by a fed-batch culture in a Jar Fermenter (MBC-3, Sanki Seiki Co., Ltd.) (Tanaka et al., 2019). At 6, 12, 24, 48 and 72 h, Na2SO4 and Na2S2O3 were supplied to the culture, giving final concentrations of 20 mM and 40 mM, respectively. The total amount of l-cysteine + l-cystine in the medium was determined by the Gaitonde method (Gaitonde, 1967). A standard curve was drawn using l-cysteine (033-05272, Fujifilm Wako Pure Chemical Co.).
Fig. 3. Transcription activities of CysB regulons do not reflect their transcript levels in ΔrppH strain.

The reporter activities of CysB regulons, sbp, cysPUWA, yeeE, and cysJIIH were examined using the pLux system as described in Fig. 1C (means ± SE, n = 3). As a non-CysB-regulon control, cysM was used. A. The promoter activities in ΔrppH cells were plotted as a relative value to that in wild-type cells in M9 medium. B. Effect of thiosulfate on promoter activity was examined. The chemiluminescence intensity from cells incubated in the presence of thiosulfate was plotted as a fold change to that in the absence of thiosulfate. Open bars, wild-type; closed bars, ΔrppH

Results

Thiosulfate selectively reduces expression of sulfur-related genes

To examine effect of thiosulfate on gene expression, microarray analysis was carried out for wild-type and ΔrppH strains in thiosulfate-containing M9 (M9+TS) medium. In wild-type cells, mRNA levels of many sulfur assimilation enzymes were clearly reduced after medium change from M9, which contains sulfate as a sole sulfur source, to M9+TS (Fig. 1A, open bars). Thiosulfate seemed to selectively down-regulate expression of the sulfur assimilation-related genes, especially genes of which expression is regulated by the transcription factor, CysB (Fig. 1B, red dot). Aiming to corroborate CysB regulation of gene expression of a sulfate/thiosulfate ABC transporter periplasmic binding protein Sbp (Hryniewicz et al., 1990), a putative sulfur transferase YeeD belonging to a TusA family and YeeE that is annotated as an inner membrane protein, we analyzed promoter activities of sbp and yeeE using the pLux system. The promoter regions of sbp and yeeE were fused to the lux operon of Photorhabdus luminescens in the pLux plasmid (Burton et al., 2010; Tobe et al., 2014). The chemiluminescence intensity from the transformants of the ΔcysB strain was monitored and compared with that of wild-type strain (Fig. 1C and Fig. S1). Like the promoter of the known CysB regulon cysJIIH, no activity of sbp and yeeE promoters was detected, suggesting that transcription of sbp, yeeE and yeeD genes is under control of CysB.

Microarray analysis indicated that, in ΔrppH cells grown in M9 medium, mRNA levels of almost 13% of genes including unknown function genes were 1.5-fold or higher than that in wild-type cells (Fig. 2A, closed bars). The results are consistent with the reports by the Belasco’s group that RppH triggers mRNA degradation (Deana et al., 2008). In their reports, expression of genes encoding yeeE, yeeD and flhY was significantly augmented in ΔrppH cells (Deana et al., 2008; Luciano et al., 2012). Our attempt discovered that, in addition to the genes, mRNA levels of cysD, cysJ and cysM genes; encoding sulfate adenyllyltransferase, sulfite reductase and cysteine synthase B, respectively were distinctly higher in ΔrppH cells as in wild-type cells (Fig. 2B). Unexpectedly, mRNA level of the cysE gene was dramatically decreased. Transcript levels of genes belonging to the CysB regulon in M9+TS medium were extremely lower than that in the absence of thiosulfate, also in the ΔrppH strain (Fig. 1A), contrary to our expectations that disruption of the rppH gene recovered the mRNA levels. The evidence suggested that the effect of thiosulfate on expression of the genes is predominant over the function of RppH.

Transcription activities of CysB regulon genes do not reflect their mRNA levels in the ΔrppH strain

Since transcriptional control is the most important factor to regulate mRNA level, transcription activities of CysB regulons were examined by a reporter assay using the pLux system. Promoter activity of the cysPUWA operon was enhanced in ΔrppH cells in comparison with that in wild-type cells (Fig. 3A), whereas no significant difference in mRNA levels of CysP, CysU, CysW and CysA was observed between ΔrppH and wild-type cells (Fig. 2B). Conversely, the promoter of the sbp gene, of which transcript level was showed no change, was clearly suppressed in ΔrppH cells. Furthermore, the rppH gene disruption increased mRNA levels of CysJ, CysM and YeeE with no significant increment of their promoter activity, suggesting post-transcriptional control by RppH-dependent mRNA degradation. Furthermore, as shown in Fig. 3B, the assay confirmed thiosulfate-induced repression of promoter activities of the CysB regulons in wild-type cells (open bars) consistent with their mRNA levels (Fig. 1A). Intriguingly, thiosulfate-induced reduction of promoter activities of sbp and yeeE in wild-type cells were recuperated in ΔrppH cells (Fig. 3B, closed bars vs. open bars). Hence, destruction of the rppH gene seemed to mitigate thiosulfate-induced down-regulation of CysB activity, whereas the recovered activities appeared not to reflect their mRNA levels.

The ΔrppH strain preferentially utilizes thiosulfate, possibly owing to a thiosulfate transporter YeeE

We further inquired sulfur metabolism in the ΔrppH strain. First, sulfate uptake was examined using [35S]-labeled Na2SO4. Wild-type and ΔrppH cells cultured in M9 medium until log-phase were incubated in 1 µM [35S]-


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labeled Na$_2$SO$_4$-containing KPM+glucose solution and the residual radioactivity in cells was monitored (Fig. 4A, solid lines). Uptake rate of $^{35}$SO$_4^{2-}$ into $\Delta$rppH cells (red closed circles) was almost half of that into wild-type cells (black closed circles). The SO$_4^{2-}$ uptake assay was also carried out in the presence of thiosulfate. M9-cultured cells were incubated in 1 µM [$^{35}$S]-Na$_2$SO$_4$- and 1 µM Na$_2$S$_2$O$_3$-containing KPM+glucose solution (Fig. 4A, dotted lines). Thiosulfate obviously attenuated the $^{35}$SO$_4^{2-}$ uptake rate into both wild-type cells (black open circles) and $\Delta$rppH cells (red open circles). In addition, intracellular content of thiosulfate was measured by the “Sulfur Index” analysis (Kawano et al., 2015b; Morikawa et al., 2012). Log-phase cells in M9 medium were incubated in M9+TS medium and thiosulfate content in cells was monitored for 15 min after a medium change to M9+TS medium (means ± SD, n = 3). Black, wild-type; red, $\Delta$rppH. C. YeeE and YeeD contributes to uptake of thiosulfate. Strains with additional mutation of either yeeE or yeeD to the $\Delta$cysA and wild-type were grown in the presence of thiosulfate as a sole sulfur source (M9-SO$_4^{2-}$+TS). Growth was monitored as OD$_{660}$ for 24 h. Open marks, wild-type background; closed marks, $\Delta$cysA background; red squares, $\Delta$yeeE; blue triangles, $\Delta$yeeD.

Fig. 4. The $\Delta$rppH strain preferentially utilizes thiosulfate, possibly owing to a thiosulfate transporter YeeE.

A. Sulfate uptake activity was examined by the tracer experiment using [$^{35}$S]-labeled SO$_4^{2-}$. Sulfate import was defined as incorporation of radioactivity into cells. Black, wild-type; red, $\Delta$rppH. Solid lines, in the absence of thiosulfate; dotted lines, in the presence of thiosulfate. B. Intracellular content of thiosulfate was monitored for 15 min after a medium change to M9+TS medium (means ± SD, n = 3). Black, wild-type, red, $\Delta$rppH. C. YeeE and YeeD contributes to uptake of thiosulfate. Strains with additional mutation of either yeeE or yeeD to the $\Delta$cysA and wild-type were grown in the presence of thiosulfate as a sole sulfur source (M9-SO$_4^{2-}$+TS). Growth was monitored as OD$_{660}$ for 24 h. Open marks, wild-type background; closed marks, $\Delta$cysA background; red squares, $\Delta$yeeE; blue triangles, $\Delta$yeeD.

The ABC transporter complex that is composed of CysU, CysW and CysA cooperates for uptake of inorganic sulfur compounds with the sulfate/thiosulfate-binding proteins CysP and Sbp (see figure 1 in Nakatani et al. (2012)). Expression of these genes was significantly attenuated in the presence of thiosulfate in both wild-type and $\Delta$rppH strains (Fig. 1A). As shown in Fig. 4C, the ABC transporter-deficient E. coli strain ($\Delta$cysA) was able to grow in the presence of thiosulfate as a sole sulfur source (closed black circles). Conversely, additional disruption of either the yeeE or yeeD gene entirely inhibited growth in the medium without sulfate, suggesting that both YeeE and YeeD are involved in uptake of thiosulfate. Since transcript levels of yeeE and yeeD genes, especially yeeE, were enlarged in the $\Delta$rppH strain (Fig. 2B; Deana et al., 2008; Luciano et al., 2012), YeeE and/or YeeD are/are presumably responsible for the transient increase of intracellular thiosulfate level in the strain (Fig. 4B).

Disruption of the rppH gene augments L-cysteine biosynthesis

The findings that RppH influenced uptake of sulfate and thiosulfate prompted us to assess intracellular content of
l-cysteine. The medium change from M9 to M9+TS induced intracellular accumulation of l-cysteine in ΔrppH cells but not in wild-type cells (Fig. 5A).

An l-cysteine-overproducing E. coli strain has been constructed by transformation with pDES plasmid that reinforces O-acetyl-l-serine production and export of l-cysteine (Daßler et al., 2000; Denk and Böck, 1987). Thus, we challenged l-cysteine production using the strains with additional disruption of the rppH gene (Fig. 5B). The destruction of the rppH gene improved l-cysteine/l-cystine accumulation in the medium in the control strain (ΔrppH vs. cont.). In addition, the effect of the rppH gene disruption on l-cysteine production was also observed in the ΔyciW background (ΔrppH ΔyciW vs. rppHWT ΔyciW). In final, we succeeded in establishing the improved l-cysteine-hyperproducing strain by additional mutations, ΔrppH ΔyciW, where we obtained ca. 2 g of l-cysteine/l-cystine per 1 L medium—the new strain was able to produce double the amount of l-cysteine, in comparison with the original strain (ΔrppH ΔyciW vs. WT).

**Discussion**

Fermentative production of l-cysteine has been challenged; e.g. (1) introducing feedback inhibition-insensitive mutation into CysE (Denk and Böck, 1987; Kai et al., 2006), (2) destruction of genes encoding l-cysteine degradation enzymes (Collins and Monty, 1973; Guarneros and Ortega, 1970; Kredich et al., 1973) and (3) reinforcement of l-cysteine export (Daßler et al., 2000; Wiriyathanawudhiwong et al., 2009). It was also demonstrated that disruption of the yciW gene encoding a putative oxidoreductase further promotes l-cysteine production (Kawano et al., 2015a, b). In addition, thiosulfate is a beneficial sulfur source for the fermentative production of l-cysteine in E. coli. However, thiosulfate strongly reduced the expression levels of genes belonging to the CysB regulon (Fig. 1A, open bars) and sulfate uptake (Fig. 4A, dotted lines). The findings seem consistent with the observation that a strain lacking thiosulfate-specific l-cysteine synthase CysM hardly produces l-cysteine in M9+TS medium (Nakatani et al., 2012), even if the medium contains as much sulfate as in M9 medium. In this study, we have, therefore, focused on mRNA metabolism, especially mRNA pyrophosphohydrolase RppH for increment of gene expression for l-cysteine production.

There were two reasons for applying the rppH gene mutation to the system of l-cysteine fermentative production. The first was that ΔrppH cells showed a potential to enhance thiosulfate uptake (Fig. 4B); and the second was that even in the presence of thiosulfate, the system with additional disruption of the rppH gene is presumably capable of maintaining the high transcript levels of CysB-regulated genes, since l-cysteine overproduction in the system is partly caused by constitutive activation of the transcription of genes for l-cysteine biosynthesis from sulfate. Eventually, the novel approach using a strain without a trigger of mRNA degradation RppH succeeded in raising the fermentative production of l-cysteine (Fig. 5B).

The microarray results indicated that, in M9 medium, transcript levels of CysB-regulated genes in ΔrppH cells are equal to or higher than that in wild-type cells (Fig. 2B). In addition, the ΔrppH strain contained a larger amount of l-cysteine in its cells, compared with that in wild-type cells, in the presence of thiosulfate (Fig. 5A). Thus, RppH certainly contributes to the control of l-cysteine production. Interestingly, the ΔrppH strain appeared to preferentially utilize thiosulfate rather than sulfate (Figs. 4A and 4B). The ABC transporter complex (CysU, CysW and CysA) cooperates with sulfate/thiosulfate-binding proteins CysP and Sbp for an inorganic sulfur compound importer, whereas the E. coli strain without the transporter complexes was able to utilize thiosulfate as a sole sulfur source, because of YeeE and YeeD (Fig. 4C). Thus, we propose that yeeE and yeeD are assigned as genes for thiosulfate uptake (tsuA and tsuB). Probably, TsuA/YeeE functions as a thiosulfate transporter, since TsuB/YeeD is not a membrane protein but a cytosolic sulfate transferase. In ΔrppH cells, the extremely high level of tsuAl/yeeE mRNA in M9 medium (Fig. 2B) probably enables higher activity of thiosulfate uptake, even after medium change to M9+TS, and causes increase of intrac-
cellular thiosulfate level. Additionally, the ΔrppH strain, even in the absence of thiosulfate, showed lower sulfate import activity (Fig. 4A, red closed circles vs. black closed circles) without any significant alteration of mRNA level for the transporters (Fig. 2B) in comparison with the wild-type strain. These findings imply a post-transcriptional regulation of the sulfate uptake.

Conclusions

In M9 medium containing sulfate as a sole sulfur source, levels of mRNAs under control of the transcription factor CysB were equal to or higher than that in the ΔrppH strain. By contrast, disruption of the rppH gene was not able to overcome thiosulfate-induced attenuation of the mRNAs, presumably owing to CysB inactivation through the higher level of L-cysteine in cells. Nevertheless, additional destruction of the rppH gene to the conventional L-cysteine fermentative production system successfully raised L-cysteine/L-cystine content in the medium, possibly because the regulation of CysB was disturbed, i.e. constitutively active, in the system. Furthermore, exceptionally enlarged expression of the thiosulfate transporter TsuA/YeeE in the thiosulfate-containing medium probably contributes to reinforce L-cysteine fermentative production in the ΔrppH strain.

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Deana, A., Ceselniak, H., and Belasco, J. G. (2008) The bacterial enzyme RppH triggers messenger RNA degradation by S’ pyrophosphate removal. Nature, 451, 355–359; doi:10.1038/nature06475. Denk, D. and Böck, A. (1987) L-cysteine biosynthesis in Escherichia coli: nucleotide sequence and expression of the serine acetyltransferase (CysE) gene from the wild-type and a cysteine-excreting mutant. J. Gen. Microbiol., 133, 515–525; doi.org/10.1099/00221287-133-3-515. Flavin, M. and Slaughter, C. (1965) Synthesis of succinic ester of homoserine a new intermediate in bacterial biosynthesis of methionine. Biochemistry, 4, 1370–1375; doi:10.1021/bi00883a022. Foley, P. L., Isich, P.-K., Luciano, D. J., and Belasco, J. G. (2015) Specificity and evolutionary conservation of the Escherichia coli RNA Pyrophosphohydrolase RppH. J. Biol. Chem., 290, 9478–9486; doi:10.1074/jbc.M114.634659. Gaitonde, M. K. (1967) A spectrophotometric method for direct determination of cysteine in presence of other naturally occurring amino acids. Biochem. J., 104, 627–633; doi:10.1042/bj1040627. Guarnieros, G. and Ortega, M. V. (1970) Cysteine desulfhydrase activity of Salmonella typhimurium and Escherichia coli. Bioch. Biophys. Acta, 198, 132–142; doi:10.1016/0005-2744(70)90041-0. Guédon, E. and Martín-Verastraete, I. (2006) Cysteine metabolism and its regulation in bacteria. In Amino Acid Biosynthesis—Pathways, Regulation and Metabolic Engineering, ed. by Wendisch, V. F., Microbiology Monographs, Vol 5, Springer, Berlin, Heidelberg, pp. 195–218; doi:10.1007/7171_2006_060. Hryniwicz, M., Sirko, A., Palucha, A., Böck, A., and Hulanicka, D. (1990) Sulfate and thiosulfate transport in Escherichia coli K-12: identification of a gene encoding a novel protein involved in thioulate binding. J. Bacteriol., 172, 3338–3366; doi:10.1128/jb.172.6.3358-3366.1990. Hsieh, P.-K., Richards, J., Liu, Q., and Belasco, J. G. (2013) Specificity of RppH-dependent RNA degradation in Bacillus subtilis. Proc. Nat. Acad. Sci. USA, 110, 8864–8869; doi:10.1073/pnas.1222570110. Kai, Y., Kashiwagi, T., Ishikawa, K., Ziyatdinov, M. K., Redkina, E. I. et al. (2006) Engineering of Escherichia coli ΔserE O-acetyltransferase on the basis of crystal structure: desensitization to feedback inhibition by L-cysteine. Protein Engineering Design & Selection, 19, 163–167; doi:10.1093/protein/gj015. Kawano, Y., Ohtsu, I., Takumi, K., Tamakoshi, A., Nonaka, G. et al. (2015a) Enhancement of L-cysteine production by disruption of yciW in Escherichia coli. J. Biosci. Bioeng., 119, 176–179; doi:10.1016/j.jbiosc.2014.07.006. Kawano, Y., Ohtsu, I., Tamakoshi, A., Shiroyma, M., Tsuruoka, A. et al. (2015b) Involvement of the yciW gene in L-cysteine and L-methionine metabolism in Escherichia coli. J. Biosci. Bioeng., 119, 310–313; doi:10.1016/j.jbiosc.2014.08.012. Kawano, Y., Suzuki, K., and Ohtsu, I. (2018) Current understanding of sulfur assimilation metabolism to biosynthesize L-cysteine and recent progress of its fermentative overproduction in microorganisms. Appl. Microbiol. Biotechnol., 102, 8203–8211; doi:10.1007/s00253-018-9246-4. Kredich, N. M. (1992) The molecular basis for positive regulation of cys promoters in Salmonella typhimurium and Escherichia coli. Mol. Microbiol., 6, 2747–2757; doi:10.1111/j.1365-2958.1992.tb01453.x. Kredich, N. M. and Tomkins, G. M. (1966) Enzymic synthesis of L-cysteine in Escherichia coli and Salmonella typhimurium. J. Biol. Chem., 241, 4955–4965. Kredich, N. M., Foote, L. J., and Keenan, B. S. (1973) The stoichiometry and kinetics of inducible cysteine desulfhydrase from Salmonella typhimurium. J. Biol. Chem., 248, 6187–6196. Luciano, D. J., Hui, M. P., Deana, A., Foley, P. L., Belasco, K. J. et al. (2012) Differential control of the rate of 5′-end dependent mRNA degradation in Escherichia coli J. Bacteriol., 194, 6233–6239; doi:10.1128/jb.01223-12. Mackie, G. A. (2013) RNase E at the interface of bacterial RNA processing and decay. Nat. Rev. Microbiol., 11, 45–57; doi:10.1038/nrmicro2930. Morikawa, T., Kajimura, M., Nakamura, T., Hishiki, T., Nakanishi, T. et al. (2012) Hypoxic regulation of the cerebral microcirculation is mediated by a carbon monoxide-sensitive hydrogen sulfide pathway. Proc. Nat. Acad. Sci. USA, 109, 1293–1298; doi:10.1073/pnas.1119658109.
Nakatani, T., Ohtsu, I., Nonaka, G., Wiriyathanawudhiwong, N., Morigasaki, S. et al. (2012) Enhancement of thioredoxin/glutaredoxin-mediated l-cysteine synthesis from S-sulfocysteine increases l-cysteine production in Escherichia coli. Microb. Cell Factories, 11, 62; doi:10.1186/1475-2859-11-62.

Ohtsu, I., Wiriyathanawudhiwong, N., Morigasaki, S., Nakatani, T., Kadokura, H. et al. (2010) The L-cysteine/L-cystine shuttle system provides reducing equivalents to the periplasm in Escherichia coli. J. Biol. Chem., 285, 17479–17487; doi:10.1074/jbc.M109.081356.

Ostrowski, J. and Kredich, N. M. (1989) Molecular characterization of the cysJIH promoters of Salmonella typhimurium and Escherichia coli: Regulation by cysB protein and N-acetyl-l-serine. J. Bacteriol., 171, 130–140; doi:10.1128/jb.171.1.130-140.1989.

Ostrowski, J. and Kredich, N. M. (1990) In vitro interactions of CysB protein with the cysJIH promoter of Salmonella typhimurium: Inhibitory effects of sulfide. J. Bacteriol., 172, 779–785; doi:10.1128/jb.172.2.779-785.1990.

Park, S. and Imlay, J. A. (2003) High levels of intracellular cysteine promote oxidative DNA damage by driving the Fenton reaction. J. Bacteriol., 185, 1942–1950; doi:10.1128/jb.185.6.1942-1950.2003.

Piton, J., Larue, V., Thillier, Y., Dorléans, A., Pellegrini, O. et al. (2013) Bacillus subtilis RNA deprotection enzyme RppH recognizes guanosine in the second position of its substrates. Proc. Nat. Acad. Sci. USA, 110, 8858–8863; doi:10.1073/pnas.1221510110.

Richards, J., Liu, Q., Pellegrini, O., Celesnik, H., Yao, S. et al. (2011) An RNA pyrophosphohydrolase triggers 5′-exonucleolytic degradation of mRNA in Bacillus subtilis. Mol. Cell, 43, 940–949; doi:10.1016/j.molcel.2011.07.023.

Shimada, T., Tanaka, K., and Ishihama, A. (2016) Transcription factor DecR (YbaO) controls detoxification of l-cysteine in Escherichia coli. Microbiology-Sgm, 162, 1698–1707; doi:10.1099/mic.0.000337.

Sirko, A. E., Zatyka, M., and Hulanicka, M. D. (1987) Identification of the Escherichia coli cysM gene encoding O-acetylserylserine sulfhydrylase B by cloning with mini-Mu-lac containing a plasmid replicon. J. Gen. Microbiol., 133, 2719–2725; doi:10.1099/00221287-133-10-2719.

Tanaka, N., Kawano, Y., Sato, Y., Dairi, T., and Ohtsu, I. (2019) Gram-scale fermentative production of ergothioneine driven by overproduction of cysteine in Escherichia coli. Sci. Rept., 9, 1895; doi:10.1038/s41598-018-38382-w.

Tobe, T., Yen, H., Takahashi, H., Kagayama, Y., Ogasawara, N. et al. (2014) Antisense transcription regulates the expression of the enterohemorrhagic Escherichia coli virulence regulatory gene ler in response to the intracellular iron concentration. PLoS One, 9, e101582; doi:10.1371/journal.pone.0101582.

van der Ploeg, J. R., Eichhorn, E., and Leisinger, T. (2001) Sulfonate-sulfur metabolism and its regulation in Escherichia coli. Arch. Microbiol., 176, 1–8; doi:10.1007/s002030100298.

Vasilyev, N. and Serganov, A. (2015) Structures of RNA complexes with the Escherichia coli RNA pyrophosphohydrolase RppH unveil the basis for specific 5′-end-dependent mRNA decay. J. Biol. Chem., 290, 9487–9499; doi:10.1074/jbc.M114.634824.

Wiriyathanawudhiwong, N., Ohtsu, I., Li, Z.-D., Mori, H., and Takagi, H. (2009) The outer membrane ToIC is involved in cysteine tolerance and overproduction in Escherichia coli. Appl. Microbiol. Biotechnol., 81, 903–913; doi:10.1007/s00253-008-1686-9.