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Regulatory Mechanism of Lysine Biosynthetic Genes in *Escherichia coli*

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In *Escherichia coli*, L-lysine biosynthetic pathway is composed of nine enzymatic reactions. It has been well established that most of the lysine biosynthetic genes are regulated by the lysine availability, even though they are all scattered around the chromosome without forming any multigenic operon structure. However, no transcriptional regulatory mechanism has been identified except for the activation of *lysA* gene by the LysR. In this study, changes in transcriptome profiles of wild type cells and *lysR* deletion mutant cells grown in the absence or presence of lysine were investigated by using DNA microarray technique. Microarray data analysis revealed three groups of genes whose expression varies depending on the availability of lysine or LysR or both. To further examine the regulatory patterns of lysine biosynthetic genes, *lacZ* operon fusions were constructed and their expression was measured under various conditions. Obtained results strongly suggest that there is an additional regulatory mechanism which senses the lysine availability and coordinates gene expression.

Introduction

L-Lysine is synthesized from aspartate through nine enzymatic steps in *Escherichia coli*. The lysine biosynthetic genes in *E. coli* are widely scattered over the chromosome without forming any multigenic operon (Fig. 1). Most of these genes are regulated at the transcriptional level by lysine suggesting the existence of a regulatory mechanism to sense the lysine availability and to alter the expression of related genes. Either by direct measurement of enzymatic activities or by analysis of the expression patterns of operon fusions between the promoter of a lysine gene and the *lacZ* reporter gene, it has been shown that *lysC, asd, dapB, dapD,* and *lysA* genes vary their expression depending on the intracellular concentration of lysine (3). However, the *lysA* gene encoding the diaminopimelate decarboxylase is the only example in which a transcriptional factor has been identified. The expression of this gene is activated by diaminopimelate but repressed by lysine. The *lysR* gene which is located next to *lysA* gene and transcribed divergently has been pointed to encode a positive regulator LysR. It has been suggested based on the genetic evidence that the LysR charged with diaminopimelate binds to the intergenic region between *lysA*

and *lysR*, causing activation of the *lysA* transcription and repression of the *lysR* transcription. The molecular machinery sensing variations of lysine concentration and modulating gene expression accordingly remains to be discovered.

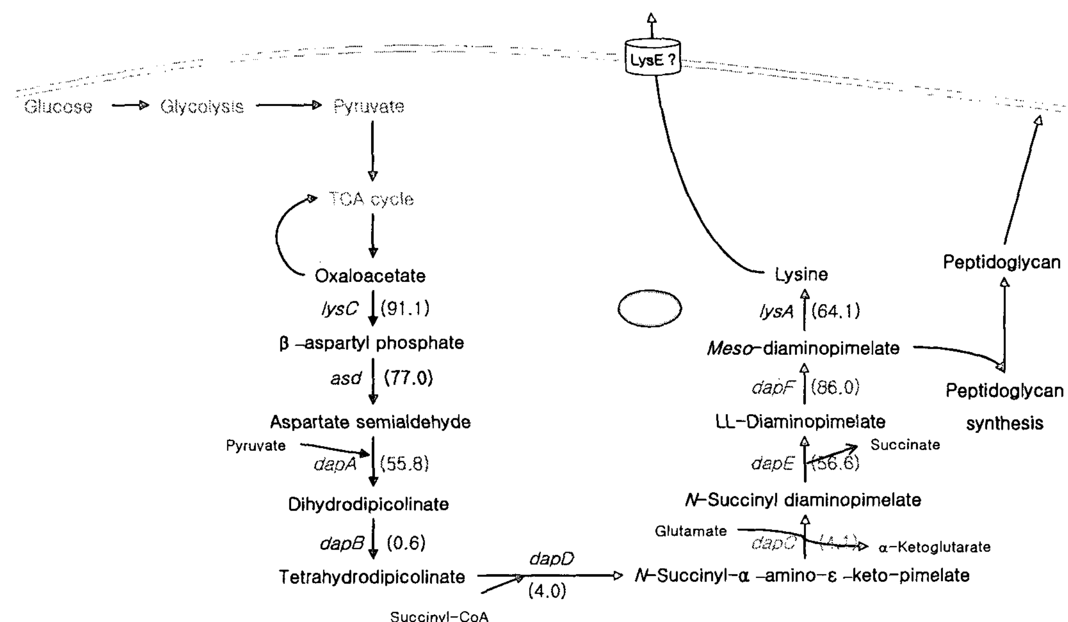

Fig. 1. Lysine biosynthetic pathway in *Escherichia coli.*

Materials and Methods

Strains and growth conditions. The *E. coli* strain W3110 and its derivatives were used for this study. For routine growth of cultures, L-agar and L-broth were used. Ampicillin, kanamycin, or chloramphenicol, when required, was added to the medium at a final concentration of 50 mg/ml, 50 mg/ml, or 20 mg/ml, respectively. For selection against *sacB*, LB medium was supplemented with sucrose to a final concentration of 5% (wt/vol). MacConkey medium (DIFCO) was used to confirm the phenotype of $\Delta lacZ$ strain. For DNA microarray analysis and β-galactosidase assay, cells were cultured in the M9 minimal medium (Sigma-Aldrich) containing 0.4% glucose. When required, 0.1 % Casamino acids or 20 mM L-lysine was added into the minimal medium.

Total RNA preparation, probe synthesis and hybridization. Total RNA was extracted from exponentially growing cultures by hot phenol method (2) and purified with RNeasy mini kit (Qiagen) and RNase-Free DNase Set (Qiagen). Entire microarray experiments from cDNA synthesis to hybridization were performed using 3DNA Array 350RP^{TM} (Genisphere) and TwinChip *E. coli*-6k (Digital Genomics, Seoul, Korea, http://www.digital-genomics.co.kr/EnglishPage/product/oligo.htm#2) as suppliers' protocol. After hybridization the slide was scanned using GenePix 4000B microarray scanner and analyzed with GenePix software (Axon Instruments, Union City, CA). Logged gene expression ratios were normalized by LOWESS regression.

Data analysis. Duplicate RNA samples were obtained for each test substance. cDNA microarray analysis was performed twice for each RNA sample. The fluorescent intensity of each spot was calculated by local median background subtraction. We used the robust scatter-plot smoother LOWESS function to perform intensity dependent normalization for the gene expression. Significance Analysis of Microarray was performed for the selection of the genes with significant gene expression changes. The statistical significance of the differential expression of any genes was assessed by computing a q-value (the lowest false discovery rate at which the gene is called significant) for each gene.

Construction of mutant strains. To construct a Δ*lysR*::kan^R strain and a Δ*lacZ* strain, flanking sequences were PCR-amplified and cloned into the plasmid pKO3. Resulting recombinant plasmids were transformed into strain W3110 to replace the corresponding wild type allele by homologous recombination as described previously (1).

Construction of promoter-*lacZ* fusion strains. To construct operon fusion strains carrying promoter sequence of various lysine biosynthetic genes fused to the promoterless *lacZ* gene, DNA fragments including the promoter and a part of coding sequence of each target gene were PCR amplified and cloned into the *lacZ* operon fusion vector pRS415 (5). Each promoter-*lacZ* fusions were then transferred to the λ transducing phage λRS45 (5). Lysates with high titers of recombinant lambda phages were used to lysogenize strain KOS4 (Δ*lacZ*), and single lysogens were selected. The Δ*lysR*::kan^R allele was P1 transduced into each reporter fusion strain. Cells were cultured in M9 glucose minimal medium with or without 20 mM L-lysine. β-Galactosidase activity was measured as described previously (5).

Results and Discussion

Transcriptome profiling of cells cultured in the absence or presence of lysine

To analyze the effects of lysine availability on genome-wide gene expression, transcriptome profiles of *E. coli* W3110 strain cultured on minimal medium in the absence or presence of L-lysine were analyzed. Out of 4,289 genes analyzed, 136 genes exhibited changes in expression level of more than 2-fold upon addition of lysine into the culture medium (Table 1). Of these 136 genes, 64 genes were upregulated and most of them were involved in the amino acid biosynthesis, cell envelop, central intermediate metabolism, energy metabolism, folding and ushering proteins, global regulatory functions, transport or binding proteins. However, the remaining 72 genes were downregulated and most of them were associated with amino acid biosynthesis, macromolecule synthesis and modification, nucleotide biosynthesis, ribosome constituents, transport or binding proteins. The most severely affected gene was the *lysC* gene encoding the aspartokinase III and was downregulated more than 7-fold. Interestingly, the lysine specific permease gene, *lysP* was also downregulated 7-fold by lysine. However, no other lysine biosynthetic gene exhibited more than 2-fold of regulation by lysine.

It has been suggested based on the genetic evidence that LysR binds to the intergenic region between *lysA* and *lysR* genes in the presence of diaminopimelate and activates the expression of *lysA*. In contrast, LysR binding causes the repression of *lysR*. To determine the effect of *lysR* deletion on genome-wide gene

expression, we cultured a *lysR* deletion mutant strain and wild type strain in the presence of lysine and compared their transcriptomes. It has been observed that *lysR* deletion resulted in expression changes of 65 genes (data not shown) which include 43 upregulated genes and 22 downregulated genes. The *lysA* was downregulated in *lysR* deletion strain. None of the other lysine biosynthetic genes was affected by *lysR* deletion

Table 1. Functional categories of differentially regulated genes by lysine

Function \ Condition	Wild type strain ± Lysine	
	up	down
Adaptation	htpX	
Amino acid biosynthesis	leuC, cysK, argI, asnA	lysC, serB, serC, aroH, tyrA, aroF, ilvB
Biosynthesis of cofactors, carriers	ribA, gst, grxB,	ribE, ispA, ubiH
Cell division	ftsI, groL	mrdA, mreB,
Cell envelop	lpcA, fimA, bolA, ompC, fimF	murA
Central intermediary metabolism	speG, pyrG, udp, hdhA	ispU, rfbB, thyA
Chromosome replication		secE
Degradation of small molecules	glk	atpI
Energy metabolism, carbon	cydA, ldhA, pykA, adhE	sdhD, fdx, gpsA
Fatty acid biosynthesis	cfa	fabH, accD,
Folding and ushering proteins	cbpA, dnaK	hscB, hscA
Global regulatory functions	yhbH, arcB, phoU	spoT
Macromolecule degradation	pepD, clpB	recJ
Macromolecule synthesis, modification	hupB	trmA, hisS, rfaF, dnaX, infA, mnmA, srmB, stpA, nusG, efp
Nucleotide biosynthesis	panE	pyrF, purE, purF, pyrC, hflD, purC, purH
Protection responses	slyA, sodB	yggT
Ribosome constituents		rpsT, rpmA, rpmG, rpmB
Some information, but not classifiable	mraW, cspD, yciW, sseA, gpmM, iaaA, yeeX, yqhD, yliH	yicE, yhdJ, tatC, rdgB, yadF, iscA, ygiC
Transport/binding proteins	modE, sstT, proW, fliY	lysP, metN, pheP, kdpB, uraA, kgtP
Unknown proteins, no known homologs	yaiB, ybdF, yccJ, nagZ, slyB, ygiB, smf, yjbQ, ycfP, ydiH, hdeB, yicH, uspD, yjcB, hdeA	iscR, yggH, yafK, ybeB, ybgF, ycbL, yfgM, iscX, iscS, yqgB

Expression of operon fusions

It has been reported based on gene fusion studies that lysine downregulates the expression of *lysC, asd, dapB, dapD,* and *lysA* genes (3). However, it is not possible to compare the relative expression levels of these genes, since some of the previous studies were performed by measuring the expression level of reporter fusions carried on a multi-copy plasmid. For more accurate measurement of the expression level of lysine genes, we constructed *lacZ* operon fusions to the promoter of the *lysC, asd, dapB, dapD,* and *lysA* genes and inserted as a single copy lysogen into the chromosome of the host strain. We also constructed *lacZ* operon fusions to the *lysP* and *lysR* genes. The expression of *lysC* and *lysP* genes was most dramatically repressed by lysine. Moreover, even though the extent was small, all the other operon fusions also exhibited lysine dependent repression except for the Φ(*lysR'-lacZ*). The *lysR* is constitutively expressed at very low level regardless of lysine availability.

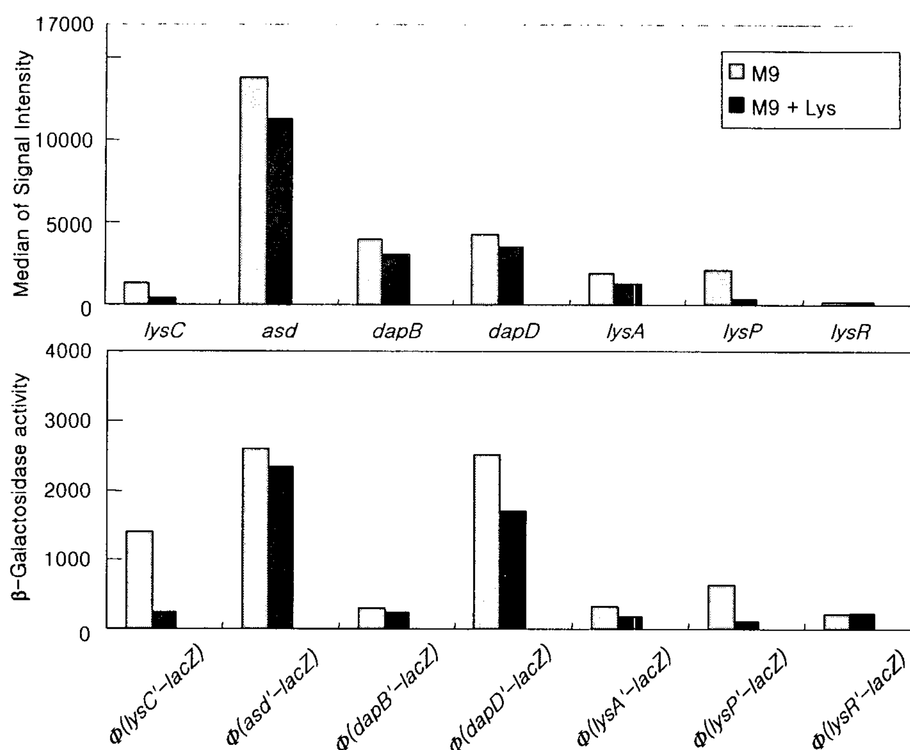

Fig. 2. Comparison of expression patterns of lysine biosynthetic genes

We also assayed the expression of all the above operon fusions in *lysR* deletion strains in the presence of lysine or lysine plus diaminaopimelate. As previously reported, the expression of *lysA* gene is activated by LysR and diaminopimelate. However, the expression of the *lysR* was not affected by *lysR* deletion or by addition of diaminopinelate into the culture medium. Interestingly, the expression of *lysC, lysP* and *asd* were increased in *lysR* deletion mutant compared to that of wild type strain.

Concluding remarks

In this study, we employed DNA microarray technique to analyze the effects of lysine availability on global gene expression in *E. coli*. Obtained results indicated that about 3% of the total ORFs in *E. coli* K-12 exhibited more than 2-fold up or down regulation by lysine. We also measured expression levels of lysine biosynthetic genes by using the conventional gene fusion technique. Interestingly, most of the lysine biosynthetic genes did not vary their expression significantly upon lysine treatment. However, the overall pattern of lysine dependent expression obtained by both methods was strikingly consistent (Fig. 2), suggesting the existence of a lysine dependent regulatory mechanism.

Acknowledgements

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