Chapter 4
Polyamine Catabolism in Prokaryotes

Hideyuki Suzuki and Shin Kurihara

Abstract Polyamines play important roles in cell growth and proliferation. In particular, these biogenic compounds are involved in the regulation of transcription and translation processes required for bacterial proliferation. Consequently, intracellular polyamine content is strictly regulated at several levels, including biosynthesis, degradation, and uptake from and excretion into the environment. In this chapter, we discuss polyamine catabolism in prokaryotes, focusing on the well-studied polyamine catabolism pathway in *Escherichia coli. E. coli* catabolizes putrescine to succinate via γ-aminobutyraldehyde (GABA) through the aminotransferase pathway or the γ-glutamylate pathway (the Puu pathway). Excess spermidine is acetylated to yield acetylspermidine, but whether this metabolite is then excreted from cells, as it is in eukaryotes, is not clear. *Pseudomonas aeruginosa* POA1, in contrast to *E. coli*, has expanded catabolic pathways to salvage cadaverine and spermidine as carbon and nitrogen sources.

Keywords Aminotransferase • Putrescine utilization pathway • Spermidine acetyltransferase • Transporter • γ-Aminobutyric acid • γ-Glutamyl intermediate • γ-Glutamylation

4.1 Introduction

The major polyamines in *Escherichia coli* cells are putrescine, spermidine, and cadaverine; this bacterium does not synthesize spermine. In eukaryotes, the lysine residue of the precursor of eukaryotic translation initiation factor 5A (elF5A) is hypusinated by the sequential reactions of two enzymes using spermidine,

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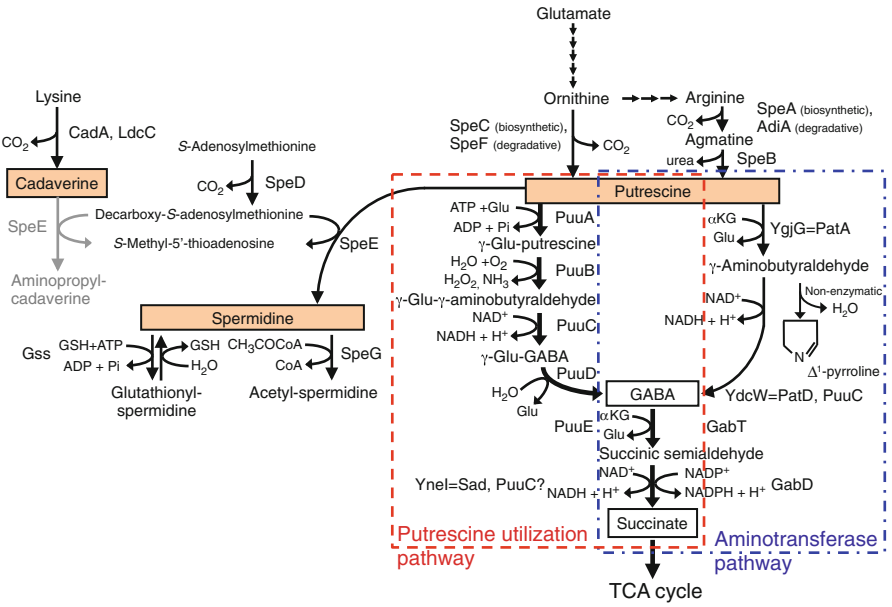

Fig. 4.1 Metabolic map of polyamines in *Escherichia coli*

and spermidine is synthesized from putrescine (see Fig. 4.1) (Park 2006; also see Chap. 10 by Park in this book). Therefore, polyamines are essential growth factors for eukaryotic cells. In contrast, an *E. coli* strain deficient in all genes for polyamine biosynthesis can grow in polyamine-free medium in an aerobic environment, albeit at reduced growth rate (Chattopadhyay et al. 2009). However, polyamines are involved in various proliferation-associated processes in bacteria, including regulation of transcription and translation, and their intracellular levels are strictly regulated by biosynthesis, degradation, and uptake from and excretion into the environment (Igarashi and Kashiwagi 2010).

E. coli synthesizes putrescine from ornithine by ornithine decarboxylases (SpeC or SpeF) or from arginine by the sequential reactions of arginine decarboxylase (SpeA) and agmatinase (SpeB) (Tabor and Tabor 1985). In addition to SpeB, the *Pseudomonas aeruginosa* PAO1 strain can convert agmatine to putrescine via *N*-carbamoylputrescine by the sequential reaction of agmatine deaminase (AguA) and *N*-carbamoylputrescine amidohydrolase (AguB) (Nakada et al. 2001). Spermidine is synthesized by the addition of propylamine to putrescine by spermidine synthetase (SpeE). Cadaverine is synthesized from lysine by lysine decarboxylases (CadA or LdcC). In spermidine deficiency, relatively large amounts of aminopropylcadaverine are synthesized as a compensatory polyamine, achieved by the addition of propylamine to cadaverine, and the resulting molecule has the same effect on polypeptide synthesis and cell growth as spermidine (Igarashi et al. 1986). An in vitro study revealed that the transfer of propylamine to cadaverine is mediated

by SpeE (Bowman et al. 1973). Hanfery et al. (2011) showed that *Campylobacter jejuni* has an alternative biosynthetic pathway of spermidine. In that pathway, not propylamine but carboxypropylamine is added from aspartate β-semialdehyde to putrescine to yield carboxyspermidine by carboxyspermidine dehydrogenase, followed by decarboxylation to yield spermidine by carboxyspermidine decarboxylase.

In this chapter, we discuss various bacterial polyamine catabolic pathways, focusing mainly on those of *E. coli*, and describe how polyamine production may be regulated and used as a bacterial feedstuff.

4.2 Putrescine Catabolic Pathways in *E. coli*

Two pathways, the aminotransferase pathway and the putrescine utilization pathway (the Puu pathway) (see Fig. 4.1), are responsible for the catabolism of putrescine to yield nitrogen and carbon sources for growth.

4.2.1 Aminotransferase Pathway

The aminotransferase pathway was first reported by Shaibe et al. (1985a, b) as a component of the arginine and ornithine catabolic networks (see Fig. 4.1). In this pathway, either arginine or ornithine is catabolized to succinate as a sole N source via putrescine and γ-aminobutyric acid (GABA). During the process, one of the amino groups of putrescine is transferred to α-ketoglutarate by putrescine aminotransferase (PatA=YgjG) to form γ-aminobutyraldehyde and glutamate. γ-Aminobutyraldehyde is further oxidized to GABA by γ-aminobutyraldehyde dehydrogenase (PatD=YdcW). The amino group of GABA is transferred to α-ketoglutarate by GABA aminotransferase (GabT) to form succinic semialdehyde and glutamate. Then, succinic semialdehyde is oxidized to succinate by succinic semialdehyde dehydrogenase (GabD). Approximate map positions of *patA* and *patD* on the genome were also reported by Shaibe et al. (1985a), although the map position of *patD* that they defined conflicts with current knowledge. PatD protein was purified in 1987 (Prieto et al. 1987). These genes, however, were not cloned until 2003 (Samsonova et al. 2003). The *ygjG* (=*patA*) gene was cloned and PatA (=YgjG) protein was purified to homogeneity from an overexpressing strain and then characterized (Samsonova et al. 2003). In the case of PatD (=YdcW), the protein was purified and the amino acid sequences of trypsin-digested peptides were compared with the *E. coli* genome database to identify its gene as *ydcW* (Samsonova et al. 2005). The k_{cat}/K_m value of YdcW against γ-aminobutyraldehyde is two orders of magnitude greater than that against butyraldehyde, indicating that γ-aminobutyraldehyde is its natural substrate. The crystal structure of YdcW (=PatD) had also been solved without knowing which pathway it involves and what is its cognate substrate (Gruez et al. 2004).

GABA released by PatD (=YdcW) is further converted to succinate for entry to the TCA cycle by the sequential reactions of GABA aminotransferase (GabT) and succinic semialdehyde dehydrogenase (GabD) (Schneider et al. 2002). Strains with deletion mutations in these genes grew normally on all tested nitrogen sources with the exception of GABA. That *gabP* gene is located next to *gabT*, which encodes a GABA transporter, and that *gabDTP* forms an operon (Maciag et al. 2011) indicates that the products of *gabDTP* genes constitute a pathway for utilization of GABA as an N source. However, a Δ*gabDT* strain is able to grow on putrescine as an N source, and still retains GABA aminotransferase and succinic semialdehyde dehydrogenase activities. Furthermore, its generation time when putrescine is used as an N source is comparable to wild-type strains (Schneider et al. 2002). Thus, there must be another pathway by which GABA generated from putrescine can be catabolized. Indeed, this is the putrescine utilization pathway that is described in Sect. 4.2.3 following.

In *P. aeruginosa* PAO1, the first enzyme of the aminotransferase pathway was reported as putrescine-pyruvate aminotransferase, which generates γ-aminobutyraldehyde and L-alanine (Lu et al. 2002; Chou et al. 2013).

4.2.2 Regulation of the Aminotransferase Pathway

The expression of *patA* gene is under the control of NtrC (nitrogen regulatory protein C) and σ^{54} (Zimmer et al. 2000; Samsonova et al. 2003; Schneider et al. 2013) and is also subjected to catabolite repression (Shaibe et al. 1985b). Because loss of both σ^{S} and σ^{54} diminished PatA activity, *patA* is transcribed with RNA polymerase not only with σ^{54} but also with σ^{S} (Schneider et al. 2013). The expression of *ydcSTUVW* (*ydcW*=*patD*) operon is regulated exceptionally by Nac (nitrogen assimilation control protein) and σ^{S} (Schneider et al. 2013).

General gene regulation by σ^{S}, σ^{54}, NtrC, and Nac can be explained as follows. In a two-component nitrogen regulatory system, the sensor histidine kinase, NtrB, senses nitrogen limitation in the medium and undergoes autophosphorylation. Then, NtrB transfers the phosphoryl group to the aspartate residue of its cognate response regulator (transcription regulator), NtrC. Phosphorylated NtrC is active, and it helps the closed complex of RNA polymerase with σ^{54} (nitrogen limitation σ factor) at the promoter to form an open complex to promote transcription initiation of genes required under nitrogen-limiting conditions (Zimmer et al. 2000). The *nac* gene is under the control of NtrC and σ^{54}, and is expressed upon nitrogen starvation. Nac activates RNA polymerase in cooperation with σ^{70} (housekeeping σ factor) to transcribe a number of operons whose products can supply the cell with ammonium or glutamate from alternative organic sources (Muse and Bender 1998). By contrast, σ^{S} is recognized as the master regulator of the general stress response, which is often accompanied by reduction or cessation of growth, and provides the cells with the ability to survive the actual stress as well as additional stresses not yet encountered (Hengge-Aronis 2002).

gabDTP consists of an operon whose $gabD_{p2}$ promoter is regulated by σ^S (Metzner et al. 2004; Maciag et al. 2011). Metzner showed that *csiD-ygaF-gabDTP* also consists of an operon and whose $csiD_p$ promoter depends on cAMP-CRP and σ^S; the operon is activated exclusively upon carbon starvation and onset of stationary phase (Metzner et al. 2004). In response to low nitrogen, the expression of *gabDTP* is upregulated by Nac and σ^{70} from an alternative promoter, $gabD_{p1}$ (Schneider et al. 2002).

4.2.3 The Putrescine Utilization Pathway (the Puu Pathway)

The other catabolic pathway of putrescine is the putrescine utilization pathway that we first reported in 2005 (Fig. 4.1) (Kurihara et al. 2005). In this pathway, extracellular putrescine is transported into the cell by transporter PuuP (its gene, *puuP*=*ycjJ*). One of the amino groups of putrescine is γ-glutamylated by γ-glutamylputrescine synthetase (PuuA) (its gene, *puuA*=*ycjK*) using ATP to generate γ-glutamylputrescine (Kurihara et al. 2008). γ-Glutamylputrescine is oxidized to γ-glutamyl-γ-aminobutyraldehyde by PuuB (its gene, *puuB*=*ordL*), which is further oxidized to γ-glutamyl-GABA by PuuC (its gene, *puuC*=*aldH*). Then, the γ-glutamyl moiety is cleaved by γ-glutamyl-GABA hydrolase (PuuD) (its gene, *puuD*=*ycjL*) to release glutamate and GABA (Kurihara et al. 2006). The amino group of GABA is transferred to α-ketoglutarate by GABA aminotransferase (PuuE) (its gene, *puuE*=*goaG*) to generate succinic semialdehyde. Then, succinic semialdehyde is oxidized to succinate by succinic semialdehyde dehydrogenase (YneI=Sad) (its gene, *yneI*=*sad*) (Kurihara et al. 2010).

Sad activity was first reported as the second succinic semialdehyde dehydrogenase, which is smaller than GabD and prefers NAD^+ as a cofactor, whereas GabD prefers $NADP^+$ (Donnelly and Cooper 1981). The Sad is induced by succinic semialdehyde, whereas *gabD* is induced by GABA coordinately with *gabT* (Donnelly and Cooper 1981). More than 25 years after that report, the gene coding Sad was first identified as *yneI* (Fuhrer et al. 2007).

PuuC was originally identified as γ-glutamyl-γ-aminobutylaldehyde dehydrogenase of the Puu pathway (Kurihara et al. 2005), but thereafter Schneider and Reitzer (2012) showed that PuuC has broad substrate specificity and utilizes not only γ-glutamyl-γ-aminobutyraldehyde, but also γ-aminobutyraldehyde and succinic semialdehyde, as substrates by comparing the activities of cell-free extracts of strains with various combinations of mutations. They also showed that PatD could be replaced by PuuC in vivo to support the growth of cells with putrescine as a sole N source. However, whether PuuC has sufficient succinic semialdehyde dehydrogenase activity in vivo to support the growth of cells with putrescine as a sole C source instead of GabD was not shown. Because *puu* genes exist as a gene cluster, it is quite likely that the natural substrate of PuuC is γ-glutamyl-γ-aminobutyraldehyde. The Δ*gabD* Δ*yneI* *aldA*$^+$ *puuC*$^+$ strain did not grow on a M9 putrescine-ammonium

chloride plate, which contains putrescine as a sole carbon source, although the Δ*gabD yneI*$^+$ *aldA*$^+$ *puuC*$^+$ strain grew on this plate at 20 °C (Kurihara et al. 2010). This observation clearly indicates that YneI, but not PuuC, supports the growth of the cells using putrescine as a sole carbon source in the absence of GabD. Although both *puu* genes and *yneI* are induced by putrescine (Kurihara et al. 2010), they are located separately on the genome at 29.3 min (Kurihara et al. 2005) and 34.7 min (Fuhrer et al. 2007), respectively.

It should be emphasized that an amino group is very reactive and one of the two amino groups of putrescine is first protected with a γ-glutamyl moiety by the reaction of PuuA at the expense of ATP before the second amino group is oxidized. Then, the γ-glutamyl moiety of γ-glutamyl-GABA is cleaved by PuuD to release GABA; the newly released amino group is further catabolized by GABA aminotransferase, PuuE. In fact, γ-aminobutyraldehyde, an intermediate of the aminotransferase pathway, is unstable, and its amino and aldehyde groups tend to form cyclic Δ^1-pyrroline nonenzymatically (Shaibe et al. 1985a). The γ-glutamylation and de-γ-glutamylation of the reactive amino group are exactly the same processes as the protection and deprotection of reactive groups performed during the chemical synthesis of some compounds. This is a rare example of the physiological role of γ-glutamylation, although there are some other examples (de Azevedo Wasch et al. 2002; Yao et al. 2011). PuuA catalyzes γ-glutamylation of putrescine to form the γ-glutamyl linkage of γ-glutamylputrescine, whereas PuuD catalyzes hydrolysis of the γ-glutamyl linkage of γ-glutamyl-GABA. If γ-glutamylputrescine, and not γ-glutamyl-GABA, were the preferred substrate of PuuD, there would be no rationale for PuuA to synthesize γ-glutamylputrescine at the expense of ATP. K_m values against γ-glutamylputrescine and γ-glutamyl-GABA were 18.5 and 2.93 mM, respectively. Also, k_{cat}/K_m values against γ-glutamylputrescine and γ-glutamyl-GABA were 23.5 and 850, respectively (Kurihara et al. 2005). These results indicate that γ-glutamyl-GABA is a far better substrate for PuuD than is γ-glutamylputrescine.

4.2.4 Regulation of the Puu Pathway

Genes coding for the Puu pathway make a gene cluster, *puuPADRCBE* (Fig. 4.2) (Kurihara et al. 2005). *puuA*, *B*, *C*, *D*, *E*, and *P* code for the members of the Puu pathway, as described in Sect. 4.2.3, and *puuR* codes for the repressor (PuuR) of *puuAP* and *puuDRCBE* operons. There are four promoters in this gene cluster (Fig. 4.2): three of them are reported to be σ^S dependent (Reitzer and Schneider 2001; Maciag et al. 2011) and the other is NtrC-σ^{54} dependent (Reitzer and Schneider 2001; Zhao et al. 2010). Maciag et al. (2011) suggested that σ^S regulates the expression of genes from arginine to succinate via putrescine through the Puu pathway.

Both *puu* genes and *yneI* are induced by putrescine (Kurihara et al. 2010). Although *puu* genes are regulated by the repressor PuuR (Kurihara et al. 2005, 2008, 2009, 2010; Nemoto et al. 2012), *yneI* is PuuR independent (Kurihara et al. 2010).

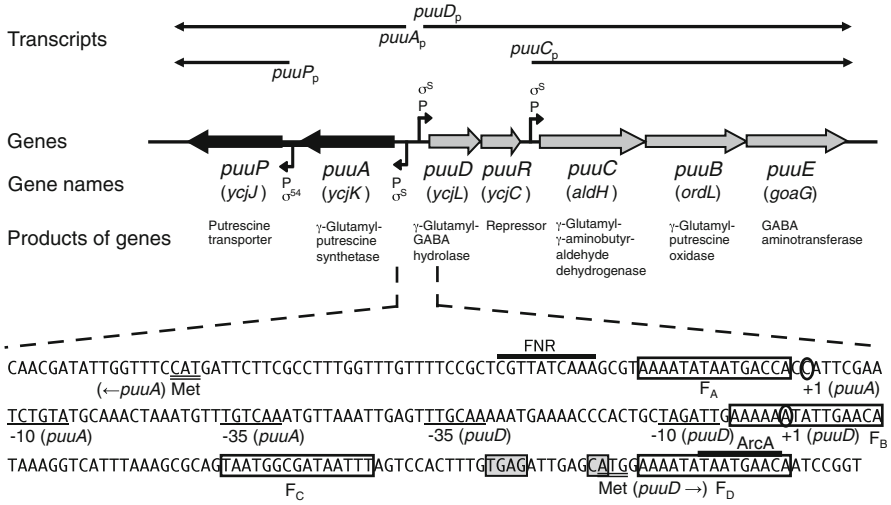

Fig. 4.2 Putrescine pathway (Puu) genes cluster of *Escherichia coli*, their transcripts, and postulated recognition sites by regulatory proteins in *puuA–puuD* intergenic region. The evidence of the transcripts and their regulation by different σ factors is described in the text. Inside the *parentheses* are the previously assigned gene names. DNA sequence shows the intergenic region and the regulatory sequences between *puuA* and *puuD*. The nucleotide sequence from position −210 to +90 from the *puuD* transcription start site is shown. The transcriptional start sites (+1) are *circled*, and the inferred −10 and −35 hexamers of the *puuA* and *puuD* promoters are *underlined*. The initiation codons of the *puuA* and *puuD* are also *double underlined* with the label "Met." The 15-bp PuuR-binding motifs are boxed and labeled F_A, F_B, F_C, and F_D, which were determined by DNase I footprint assay (Nemoto et al. 2012). A cAMP-CRP recognition site (Shimada et al. 2011) is *boxed gray*. Predicted FNR and ArcA recognition sites (Partridge et al. 2006) are shown by *thick black lines* above the sequence

Schneider and Reitzer (2012) showed that the in-frame Δ*puuA* strain, which does not cause a polar effect on *puuP*, could grow on putrescine as an N source if the enzymes of the aminotransferase pathway were present. By contrast, they revealed that Δ*puuP* strain could not grow on putrescine as an N source even though the enzymes of aminotransferase pathway are present. This finding conflicts with our result that Δ*puuA*::*kan* strain, which could not utilize putrescine as a sole C source and a sole N source, complemented the growth on putrescine with a plasmid that expresses only PuuA (Kurihara et al. 2008). PuuP is the essential putrescine transporter required for the cells to grow on putrescine as an N source (Kurihara et al. 2009; see Chap. 14 by Kurihara and Suzuki in this book). Because the *puuP* gene can also be transcribed under the control of NtrC and σ^{54} from the promoter $puuP_p$, which is separate from the promoter $puuA_p$ under the control of σ^S, its expression could be coordinated with that of the aminotransferase pathway.

We observed that the expression of *puu* genes was induced at high aeration by putrescine and reduced by either glucose, succinate, or NH_4Cl (Kurihara et al. 2005, 2006). Since then, the molecular mechanisms of regulations of *puu* genes have been elucidated. The expression of *puu* genes is repressed by PuuR, which has a

helix-turn-helix DNA-binding motif and is coded in the *puu* gene cluster; this cluster is induced in the presence of putrescine (Nemoto et al. 2012). A probe corresponding to the intergenic region between *puuA-puuD* was shifted in the presence of purified His_6-PuuR by a gel mobility shift assay, and the amount of the shifted bands was inversely proportional to the concentration of putrescine but not to that of spermidine. However, the probes corresponding to the intergenic regions between *puuA-puuP* and *puuR-puuC* were not shifted. Transcript sizes were checked, and it was confirmed that *puuAP* and *puuDRCBE* transcripts exist. DNase I footprint analysis of the intergenic region between *puuA-puuD* showed there are four PuuR-binding sites (Fig. 4.2), and Nemoto et al. (2012) proposed a consensus binding sequence that consists of 15 nucleotides with an asymmetrical recognition sequence, AAAATATAATGAACA, which is in the PuuR-binding site from 20 nucleotides (ATGGaCAATATATTGaCCAT) with an inverted repeated symmetry that was suggested by the curator of the Regulon Database (URL: http://regulondb.ccg.unam.mx/).

puu genes are repressed by the addition of glucose, and the regulatory mechanisms involved are now known. There is a cAMP-CRP recognition site between the transcription initiation site and the initiation codon of *puuD* (Fig. 4.2) (Shimada et al. 2011). Terui et al. (2014) showed that the expression of *puuR* from the $puuD_p$ promoter is positively regulated by glucose. That is, in the presence of glucose the concentration of cAMP decreases and CRP no longer binds to the cAMP–CRP recognition site in front of the initiation codon of *puuD*. This stage releases the inhibition of transcription of *puuR*, and PuuR as synthesized then represses transcription from $puuA_p$ and $puuD_p$ as a result.

Partridge et al. (2006) observed that *puu* genes are induced upon a shift from anaerobic to aerobic conditions in *E. coli*. They predicted an FNR recognition site and an ArcA recognition site in the *puuA–puuD* intergenic region and suggested the de-repression of the *puu* genes in the presence of O_2. They also showed that the supercoiling of plasmid was changed along with the shift from anaerobic to aerobic condition, but this phenomenon was delayed in the *puuA* mutant. This investigation leads to a model in which O_2 induces the Puu pathway, reduces the intracellular putrescine concentration, and causes the topological changes in DNA that influence the transcription of various genes.

Another possible regulatory mechanism of the Puu pathway is the metal-catalyzed oxidative modification of PuuA followed by proteolytic degradation. This regulatory mechanism was proposed to be one of the regulatory mechanisms of glutamine synthetase, a key enzyme of ammonium assimilation (Liaw et al. 1993). PuuA has high amino acid sequence similarity to glutamine synthetase. The enzymatic reactions catalyzed by glutamine synthetase and PuuA are also very similar in terms of the amide bond formation between the γ-carboxyl group of glutamate and ammonia, and the γ-carboxyl group of glutamate and the amino group of putrescine, respectively. Both enzymes exist as homododecamers and require Mg^{2+} or Mn^{2+} for activity (Stadtman and Ginsburg 1974; Kurihara et al. 2008). The important amino acid residues for two metal-binding sites, the glutamate and ATP-binding sites, are well conserved. It was reported that the oxidative modification of His-269 to Asn and Arg-344

to Gln induces the loss of activity of glutamine synthetase followed by increased susceptibility to proteolytic degradation (Liaw et al. 1993). Because these residues are also conserved in PuuA in addition to the similarities already mentioned, it is plausible that PuuA is also subjected to metal-catalyzed oxidative modification followed by proteolytic degradation. In fact, PuuA protein is much more unstable than many other proteins. Because PuuA is the key enzyme of the Puu pathway and the catabolism of putrescine, it is quite rational that its prompt decay allows *E. coli* to adapt to the sudden decrease of intracellular putrescine concentration.

4.3 Catabolic Pathways of Spermidine

E. coli can increase intracellular spermidine concentration by synthesizing it from putrescine or by importing it from the environment by PotABCD (Igarashi and Kashiwagi 1999). Conversely, intracellular spermidine concentration is reduced by the activity of spermidine acetyltransferase. This enzyme acetylates spermidine to form acetylspermidine by using acetyl-CoA (Fukuchi et al. 1994). In eukaryotes acetylspermidine is excreted from the cell (Gerner and Meyskens 2004), but the fate of acetylspermidine in *E. coli* is not clear. Intracellular spermidine concentration in the wild-type strains does not vary dramatically between cells grown in the presence and absence of 0.5 mM spermidine. On the other hand, the spermidine acetyltransferase-deficient (*speG*$^-$) strain markedly accumulates spermidine when it is grown in the presence of 0.5 mM spermidine, but not in the absence of spermidine (Fukuchi et al. 1995). The growth of the *speG*$^-$ strain in M9 medium was normal in the presence and absence of 0.5 mM spermidine, but its viability at the late stationary phase was greatly decreased compare to the wild type. Fukuchi et al. (1995) suggested that a decrease of various protein syntheses that included ribosome modulation factor essential for cell viability at the stationary phase was caused by the accumulation of spermidine in the *speG*$^-$ strain.

The other enzyme that may decrease the intracellular spermidine in *E. coli* is glutathionylspermidine synthetase/amidase (Gss = Gsp). Gss was first studied extensively by Bollinger et al., who found that the enzyme is bifunctional and consists of two domains that are responsible for catalysis of the reverse reactions (Bollinger et al. 1995; Kwon et al. 1997). The three-dimensional structure of Gss has been reported (Pai et al. 2006), and its role in redox regulation was studied (Chiang et al. 2010). However, there has been no report if it regulates the intracellular spermidine concentration.

P. aeruginosa PAO1 has spermidine dehydrogenase (SpdH), which cleaves spermidine into 1,3-diaminopropane and γ-aminobutyraldehyde and spermine into spermidine and 3-aminopropanaldehyde. γ-Aminobutyraldehyde is further oxidized to GABA by KauB, which corresponds to PatD, followed by catabolism to succinate by GabT and GabD. 3-Aminopropanaldehyde is oxidized to β-alanine by KauB, and subsequently catabolized to acetyl-CoA by β-alanine-pyruvate aminotransferase (BauA) and malonic semialdehyde dehydrogenase (BauB). However,

SpdH is not induced by exogenous polyamines, and the *spdH* knockout mutant grows normally on spermidine and spermine (Dasu et al. 2006). According to a database search using Blastp (Altschul et al. 1997), *E. coli* does not have a SpdH homologue. In *P. aeruginosa* PAO1, spermidine is catabolized by the *pau* pathway, which is discussed in the next section, and all seven *pauA* genes-deficient mutants cannot grow on spermidine.

4.4 Catabolic Pathways of Cadaverine

In *E. coli* a cadaverine-specific catabolic pathway has not yet been reported. Despite the lack of in vivo evidence, the activity of PatA (=YgjG) toward cadaverine is comparable to that toward putrescine (Samsonova et al. 2003), and the activity of PuuA toward cadaverine is about one third of that toward putrescine in vitro, implying that both the aminotransferase and the Puu pathways are involved in cadaverine catabolism. If this is true, then either PuuE or GabT might also use δ-aminovalerate, and either YneI or GabD could use glutamic semialdehyde as substrate. However, this requires further experimental testing. In *P. aeruginosa* PAO1, seven *pauA* genes, four *pauB* genes, one *pauC* gene, and two *pauD* genes, which correspond to *puuA, B, C,* and *D* gene of the Puu pathway, respectively, are responsible to various polyamines catabolism. Each PauA has different specificity toward each polyamine, and specific combination of *pauA* knockouts is required to abolish the utilization of specific polyamines (Yao et al. 2011). It has also been demonstrated that PauR controls *pau* promoters in response to putrescine and cadaverine (Chou et al. 2013).

In this strain, spermidine-inducible genes overlap almost completely with putrescine-inducible *pau* genes, with the exception of *pauA3B2* and *bauABCD* operons (Yao et al. 2011). PauA3 and PauB2 are involved in the catabolism of diaminopropane, generated from the aminopropyl moiety of spermidine, and BauA and BauB are involved in β-alanine catabolism as described in Sect. 4.3 (Yao et al. 2011). A single knockout mutation of the *pauA2* gene blocks growth on spermidine completely, but the mutant can grow on putrescine, cadaverine, or diaminopropane (Yao et al. 2011). This indicates that PauA2 is a spermidine-specific γ-glutamyl ligase. However, it is still unclear whether the amino group of the aminopropyl moiety or that of the aminobutyl moiety is γ-glutamylated and how the internal C–N bond is cleaved.

4.5 Future Perspectives

Can *E. coli* degrade cadaverine and spermidine as does *P. aeruginosa*? How does *P. aeruginosa* degrade spermidine through the Pau pathway? How does the intracellular putrescine concentration respond to various stresses? These are questions that should be answered in the near future.

Global warming has become a major issue, and there is a move away from fossil resources toward recyclable resources, not only as energy sources but also as chemical feedstocks. Polyamines are used as intermediate materials in the production of synthetic fibers and fabrics. At present, they are mainly produced from petroleum by industrial chemical processes, but putrescine, spermidine, and cadaverine can all be synthesized biologically from arginine, ornithine, and lysine. These amino acids are produced industrially by fermentation. To obtain a higher polyamine yield and industrialize the process, we await a more detailed understanding of polyamine metabolism in bacteria.

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