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INVITED REVIEW

Regulation of arginine biosynthesis, catabolism and transport in *Escherichia coli*

Daniel Charlier[1] · **Indra Bervoets**[1]

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Abstract
Already very early, the study of microbial arginine biosynthesis and its regulation contributed significantly to the development of new ideas and concepts. Hence, the term "repression" was proposed by Vogel (The chemical basis of heredity, The John Hopkins Press, Baltimore, 1957) (in opposition to induction) to describe the relative decrease in acetylornithinase production in *Escherichia coli* cells upon arginine supplementation, whereas the term "regulon" was coined by Maas and Clark (J Mol Biol 8:365–370, 1964) for the ensemble of arginine biosynthetic genes dispersed over the *E. coli* chromosome but all subjected to regulation by the *trans*-acting *argR* gene product. Since then, unraveling of the molecular mechanisms controlling arginine biosynthesis, catabolism, and transport in and out the cell, have revealed moonlighting activities of enzymes and transcriptional regulators that generate unexpected interconnections between at first sight totally unrelated cellular processes, and have continued to replenish scientific knowledge and stimulated creative thinking. Furthermore, arginine is much more than just a common amino acid for protein synthesis. It may also be used as sole source of nitrogen by *E. coli* and a source of nitrogen, carbon and energy by many other bacteria. It is a substrate for the synthesis of polyamines, and important for the extreme acid resistance of *E. coli*. Furthermore, the guanidino group of arginine is well suited to engage in multiple interactions involving hydrogen bonds and ionic interactions with proteins and nucleic acids. Here, we combine major historical discoveries with current state of the art knowledge on arginine biosynthesis, catabolism and transport, and especially the regulation of these processes in *E. coli*, with reference to other microorganisms.

Keywords Arginine · Lysine · Leucine · Transcriptional regulation · Protein-DNA interactions · Feedback inhibition

Importance of arginine in bacterial physiology

Arginine is not just one of the common amino acids required for protein synthesis (overall abundance of about 5% in the *Escherichia coli* proteome), the basic amino acid also plays an important role in several other aspects of cellular growth and physiology (Fig. 1). Thus, upon anaerobic growth on complex medium, arginine is important for the extreme acid resistance of *E. coli* cells (Lin et al. 1995; Gong et al. 2003; Richard and Foster 2004). In conditions of nitrogen limitation and aerobic growth, the energy and nitrogen-rich amino acid (aa) may serve as sole source of nitrogen through degradation by the arginine succinyltransferase pathway (AST) in *E. coli* and *Salmonella typhimurium* (*Salmonella enterica* serovar Typhimurium) (Schneider et al. 1998; Lu and Abdelal 1999), and as a source of nitrogen, carbon, and energy in other organisms such as *Pseudomonas aeruginosa* that disposes of additional catabolic pathways (arginine deiminase, arginine decarboxylase, and arginase) for the degradation of arginine under aerobic and anaerobic growth conditions (Tricot et al. 1991; Jann et al. 1988; Reitzer 2005; Zúñiga et al. 2002; Lu 2006). Arginine and its precursor ornithine are also substrates for the synthesis of the major polyamines putrescine and spermidine (reviewed in Charlier and Glansdorff 2004; Miller-Fleming et al. 2015; Igarashi and Kashiwagi 2018), and ornithine may serve as starting point for the synthesis of proline (Fichman et al. 2015)

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✉ Daniel Charlier
dcharlie@vub.be

[1] Research Group of Microbiology, Department of Bio-engineering Sciences, Vrije Universiteit Brussel, Pleinlaan 2, 1050 Brussels, Belgium

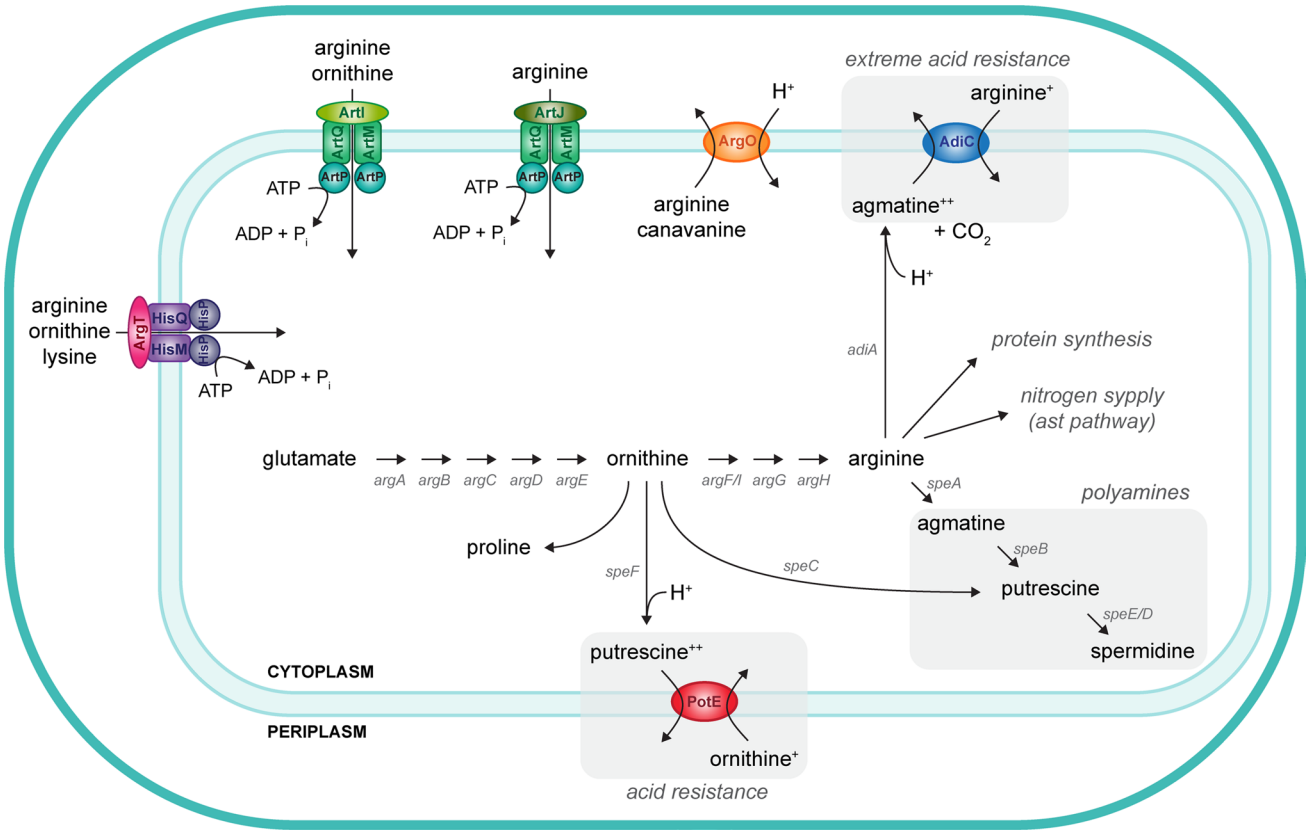

Fig. 1 Schematic representation of arginine metabolism and its import and excretion by different transport systems in *E. coli*. Biosynthesis starting from glutamate, arginine degradation via the arginine succinyl pathway (*ast*) (as source of nitrogen), decarboxylation of arginine and ornithine for the synthesis of polyamines (*speA*, *speC*) and extreme acid resistance (*adiA*, *speF*), and transport of arginine in and out the cell via ABC-type uptake systems, antiport systems for arginine–agmatine (AdiC) and ornithine–putrescine (PotE), and the *argO*-encoded export system for arginine and canavanine driven by the energy of the proton motive force are illustrated

(Fig. 1). Furthermore, with its guanidinium group that may engage in the formation of multiple hydrogen bonds and ionic interactions, the long side chain of arginine is particularly well suited to establish inter- and intramolecular interactions. On the surface of proteins, arginine residues are interesting partners in the formation of stabilizing ion networks, and as a frequent constituent of DNA-binding motifs arginine that is able to make bidentate contacts strongly contributes to the sequence specificity and strength of target site recognition through arginine-base and arginine–phosphate interactions. The interaction of arginine with the N_7 and O_6 atoms of guanine is even among the most abundant and energetically strongest interactions observed in cocrystals of protein-DNA complexes (Lustig and Jernigan 1995; Wintjens et al. 2000; Lejeune et al. 2005; Rhodes et al. 2010). Finally, it is worth mentioning that arginine is an important nutraceutical, hence the interest for the development of improved microbial production strains (Tuchman et al. 1997; Rajagopal et al. 1998; Ikeda 2003; Ikeda et al. 2009; Lu 2006; Ginesy et al. 2015).

Arginine biosynthesis

Peculiarities of the pathway in *E. coli*

Starting from glutamate, arginine is synthesized in eight enzymatic steps (Fig. 2). The first four intermediates are *N*-α-acetylated (Vogel 1970). This must avoid their cyclization and use in proline biosynthesis, where the spontaneous cyclization of γ-glutamyl semialdehyde generates Δ1-pyrroline-5-carboxylate that is further reduced to proline by Δ1-pyrroline-5-carboxylate reductase (Fichman et al. 2015). In *E. coli* and *Enterobacteriaceae* in general, arginine is synthesized through the so-called linear pathway (Cunin et al. 1986; Xu et al. 2000), in which acetylornithine is deacylated by a Co^{2+}-dependent *argE*-encoded acetylornithine deacetylase (acetylornithinase, AO) to produce ornithine and acetate (Javid-Majd and Blanchard 2000).

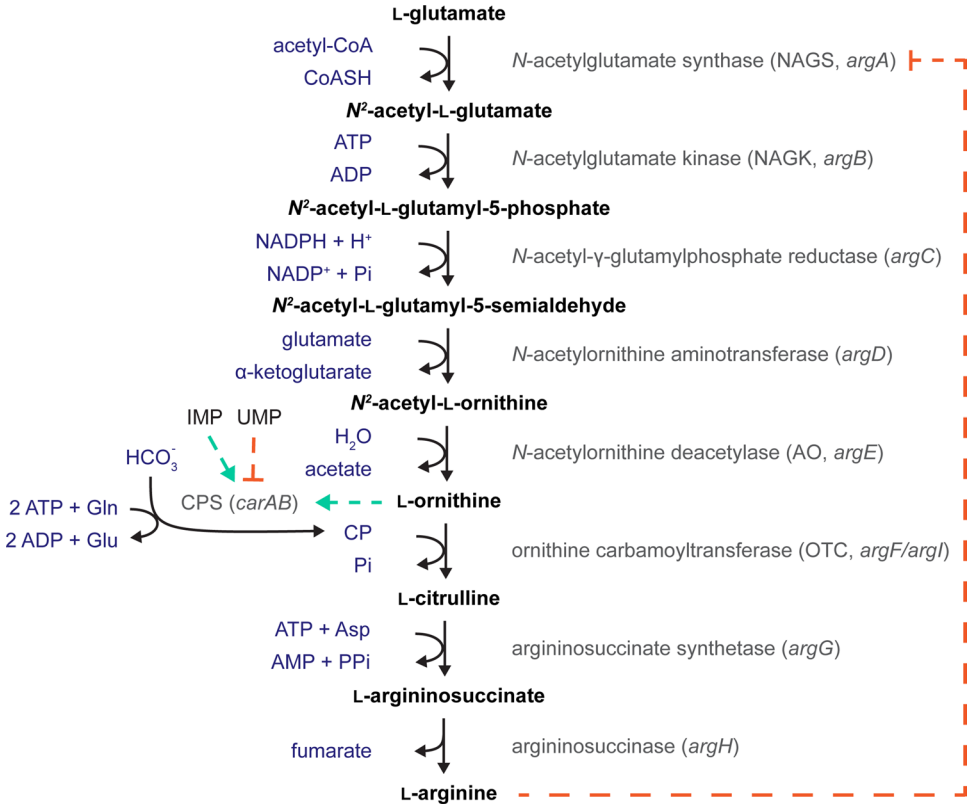

Fig. 2 Biosynthesis of arginine via the linear pathway as it occurs in *E. coli*. Feedback inhibition of *N*-acetylglutamate synthase (NAGS) by arginine is indicated with a dotted line. Carbamoylphosphate synthase activity (CPS) is activated by ornithine and IMP, and inhibited by UMP. The synthesis of all biosynthetic enzymes including CPS is repressed at the transcriptional level by arginine-bound ArgR (not indicated)

AO is homologous to succinyldiaminopimelate desuccinylase (*dapE*) of lysine biosynthesis and carboxypeptidase G2, and hence an example of enzyme recruitment (Boyen et al. 1992). In this context, it is worth noticing that *dapC*-encoding *N*-succinyl-L,L-diaminopimelate:alpha-ketoglutarate aminotransferase, another enzyme of lysine metabolism, was found to be identical to *argD*, encoding *N*-acetylornithine aminotransferase, which consequently, has a double function in the biosynthesis of arginine and lysine (Ledwige and Blanchard 1999). The extent and significance of the redundancy of aminotransferases involved in arginine and lysine biosynthesis in *E. coli* is further illustrated by the mutant and complementation studies of Lal et al. (2014).

Escherichia coli AO exhibits a broad substrate specificity and readily deacetylates among others *N*-acetylarginine, *N*-acetylhistidine, *N*-acetylglutamate-γ-semialdehyde and the toxic analogue *N*-acetylnorvaline, a capacity that has been exploited for the selection of various types of *cis*- and *trans*-acting regulatory mutations of arginine biosynthesis and the isolation of novel aminoacylase-encoding genes (Baumberg 1970; Bretcher and Baumberg 1976; Kelker and Maas 1974; Boyen et al. 1978; Charlier et al. 1978; Sakanyan et al. 1993b). The capability of using *N*-acetylglutamate-γ-semialdehyde as a substrate also explains the observed indirect phenotypic suppression of proline auxotrophs (*proA* and *proAB* mutants) by mutations inactivating *argD* (Itikawa et al. 1968; Kuo and Stocker 1969). The rationale behind this observation is as follows: *proA* and *proAB* mutants are blocked in the conversion of glutamate into glutamate-γ-semialdehyde, and *argD* mutants exhibit a leaky argnineless phenotype, likely because another transaminase can partially take over the missing function. When *proAB argD* mutants are grown in the absence of arginine, the internal arginine pool is very low. As a consequence, the whole arginine biosynthetic pathway is transcriptionally derepressed and the feedback inhibition of *N*-acetylglutamate synthase (ArgA, NAGS) lifted (see below, parts on regulation of gene expression and regulation of enzyme activity, respectively), which results in the accumulation of sufficient *N*-acetylglutamate-γ-semialdehyde that can be deacylated by the broad substrate range AO to feed the proline pathway.

Many other bacteria and fungi use the more widespread and energetically more favorable non-linear or cyclic pathway for arginine biosynthesis, in which the acetyl group of acetylornithine is recycled on glutamate by an ornithine glutamate acetyltransferase (*argJ*, OAT), as originally discovered in *Micrococcus glutamicus* (renamed *Corynebacterium glutamicum*) (Udaka and Kinoshita 1958) and later also found in the model organisms *Bacillus subtilis*, *Bacillus licheniformis*, *Bacillus stearothermophilus* (renamed *Geobacillus stearothermophilus*) and *P. aeruginosa* (Sakanyan et al. 1992, 1993a, 1996; Haas et al. 1972; Xu et al. 2007). In some bacteria and yeast species including *B. subtilis*, *G. stearothermophilis*, *Neisseiria gonorrhoeae*, *Thermotoga*

neapolitana, *Thermotoga maritima*, and *Saccharomyces cerevisiae*, a single enzyme combines both NAGS and OAT activity (Sakanyan et al. 1992, 1993a; Crabeel et al. 1997; Xu et al. 2007). These bifunctional enzymes are capable of synthesizing acetylglutamate de novo, that is with glutamate and acetyl-CoA as substrates. At first sight, this may appear surprising since there is no significant similarity between *argJ* and *argA*, but the reaction mechanism of these two enzymes is different (Dyda et al. 2000; Marc et al. 2000; Weigel et al. 2002). Interestingly, the production of active ArgJ enzymes requires posttranslational autoprocessing of the precursor protein by self-catalyzed cleavage of the peptide bond adjacent to a conserved threonine residue (Abadjieva et al. 2000; Marc et al. 2001). The latter is not only crucial for the self-splicing, but subsequent to cleavage also becomes the catalytic nucleophile at the *N*-terminus of the β-subunit of the heterooligomeric enzyme. Mono- and bifunctional OATs have been found in association with a classical *argA*-encoded NAGS (Xu et al. 2006, 2007). In the case of monofunctional OATs, as present in, e.g., *P. aeruginosa*, NAGS plays an anaplerotic role, furnishing the first molecule of *N*-acetylglutamate to initiate the otherwise cyclic pathway. In organisms harboring a NAGS and a bifunctional OAT, such as *G. stearothermophilus*, there is evidently functional redundancy between the two *N*-acetylglutamate synthase activities, but it appears that production of *N*-acetylglutamate by the OAT-dependent transacetylation is the most prominent one (Weigel et al. 2002).

Remarkably, *E. coli* K-12 possesses two genes, *argF* and *argI,* encoding ornithine carbamoyltransferases (OTC) with different properties (Glansdorff et al. 1967; Legrain et al. 1976) but ArgF and ArgI subunits (86% aa sequence identity) interact to generate four trimeric isoenzymes (Legrain et al. 1972). *argI* is the sole gene present in other *E. coli* strains and in *Enterobacteriaceae* in general (Jacoby 1971; Legrain et al. 1976). *argF* appears to be acquired by lateral gene transfer. This is underscored by the higher G + C content of *argF* as compared to *argI* (Van Vliet et al. 1984), and the observation that the gene is flanked by two IS*1* insertion sequences (Hu and Deonier 1981; York and Stodolski 1981). Hence, it has all the characteristics of a composite transposon. *E. coli argF* constitutes one of the very first convincingly documented cases of information acquisition by lateral gene transfer. OTC exhibits significant overall aa sequence similarity (35–40%) with the catalytic subunit (PyrB) of aspartate carbamoyltransferase (ATC), encoded by the *pyrBI* operon, which catalyzes a similar reaction in the pyrimidine biosynthetic pathway (Houghton et al. 1984; Van Vliet et al. 1984). Therefore, it appears that the paralogs OTC and ATC have a common origin and likely result from the duplication of an ancestral gene followed by specialization in the course of evolution. This hypothesis is underscored by sequence comparisons of OTC and ATC enzymes from a large number of diverse organisms (Labedan et al. 1999) and the observation that the fusion of the polar domain of OTC to the carboxy-terminal domain of ATC results in an active enzyme that can use aspartate but not ornithine in a carbamoylation reaction (Houghton et al. 1984).

The conversion of ornithine into citrulline by OTC requires carbamoylphosphate (CP) as a second substrate (Fig. 2). CP is also required for the conversion of aspartate into carbamoylaspartate by ATC as the first committed step in pyrimidine biosynthesis (Lipscomb and Kantrowitz 2012). Hence, it is a substrate common to both pathways (Jones et al. 1955). In contrast to most other organisms, *E. coli* and *Enterobacteriaceae* in general possess a single carbamoylphosphate synthase (CPS), encoded by the *carAB* operon, to produce all the CP (Abd-El-Al and Ingraham 1969; Piérard and Wiame 1964; Piérard et al. 1965). The de novo arginine and pyrimidine biosynthetic pathways are thus intimately interconnected and the dual role of CPS is reflected in the complex regulation of *E. coli carAB* gene expression by multiple transcription factors and effector molecules from the arginine, pyrimidine and purine biosynthetic pathways, and the allosteric control of CPS activity (see below and Charlier et al. 2018).

Regulation of de novo arginine biosynthesis

Regulation of enzyme activity: *N*-acetylglutamate synthase (NAGS) and carbamoylphosphate synthase (CPS)

In *E. coli* and other organisms that synthesize arginine via the linear pathway, NAGS appears to be the only highly regulated enzyme (besides the CP producing CPS, see below). NAGS is feedback inhibited by the end product of the pathway (Fig. 2) (50% inhibition at 20 μM L-arginine) and equally by the arginine analog O-(L-norvalyl-5)-isourea (Vyas and Maas 1963; Leisinger and Haas 1975; Marvil and Leisinger 1977; Abdelal and Nainan 1979). The importance of feedback inhibition of NAGS in regulation of arginine biosynthesis is underscored by the observation that full transcriptional repression of the arginine biosynthetic genes is rapidly achieved (within 2 min after arginine addition) but enzyme concentrations are only diluted at the rate of cell division (Caldara et al. 2006, 2008). *Escherichia coli* NAGS is a homohexameric enzyme composed of subunits comprising two domains (Marvil and Leisinger 1977). The N-terminal part that binds glutamate contains a carbamate kinase (CK) fold as present in *N*-acetylglutamate kinase (NAGK, *argB*), that catalyzes the second step of arginine biosynthesis (Ramon-Maiques et al. 2002, 2006). The C-terminal domain harbors an acetyl-CoA-binding fold as observed in the superfamily of GCN5 (a eukaryotic histone acetyltransferase)-related *N*-acetyltransferases (GNATs) (Dyda et al. 2000; Vetting et al. 2005). The reaction mechanism used by members

of this large superfamily of GNATs with representatives in all domains of life, including *E. coli* NAGS, is highly conserved and passes through the formation of a ternary complex between the enzyme and its substrates, followed by the nucleophilic attack by the amine and elimination of CoA (thus a sequential mechanism). The link between arginine and proline biosynthesis (see above) allowed the selection of various desensitized mutants of ArgA activity in *E. coli* and *S. typhimurium* in a two-step procedure, in which *argD proAB* double mutants are incubated in minimal medium without proline supplementation. This selection first resulted in the generation of slow growing genetically derepressed *argR* mutants from which *argA* feedback insensitive mutants can be obtained (Itikawa et al. 1968; Kelln and O'Donovan 1976). Desensitized *argA* mutants could equally be selected in a single step from a mutagenized culture of a triple *argD pro argR* mutant by selecting for proline excreters (Eckhardt and Leisinger 1975). Experimental perturbation and mathematical modeling indicated that allosteric feedback inhibition of NAGS is required in combination with ArgR-mediated transcriptional regulation of arginine biosynthetic enzyme production to ensure robust and controlled arginine biosynthesis (Caldara et al. 2008), as further confirmed in a recent study (Sander et al. 2019). In various combinations with mutations resulting in enhanced arginine biosynthetic enzyme activity, altered arginine transport (reduced uptake, enhanced excretion) or catabolism (e.g., *argR*, *argP*, *adiA*), arginine-desensitized *argA* mutants were used for the construction of more performant arginine producing strains of *E. coli* and other bacterial model organism including *P. aeruginosa* for industrial production (Tuchman et al. 1997; Rajagopal et al. 1998; Lu 2006; Caldara et al. 2008; Ginesy et al. 2015).

In organisms that harbor the cyclic pathway for arginine synthesis, relying on an acetyltransferase (OAT) to recycle the acetyl group of *N*-acetylornithine on glutamate, it is the kinase NAGK or the OAT itself that is feedback inhibited (Udaka 1966; Sakanyan et al. 1993a; Van de Casteele et al. 1990) and in organisms such as *P. aeruginosa* that possesses a monofunctional OAT and a NAGS, the latter is feedback inhibited as well (Haas et al. 1972). In the yeast *S. cerevisiae* that uses the cyclic pathway for arginine synthesis, the two first enzymes of the pathway, NAGS and NAGK, are feedback inhibited. Remarkably, the two enzymes associate in a complex. Furthermore, the synthase is only active when complexed with the kinase (Abadjieva et al. 2001) and the feedback inhibition of the synthase and the kinase is mutually interdependent (Pauwels et al. 2003).

In *E. coli* and related organisms, the high-energy molecule CP is synthesized from glutamine, bicarbonate, and two molecules of Mg^{2+}ATP by a single enzyme (CPS), encoded by the *carAB* operon (Piérard and Wiame 1964; Piérard et al. 1965) (Fig. 2). *Escherichia coli* CPS belongs to the class I amidotransferase family (Raushel et al. 1999) and consists of two subunits, a small *carA*-encoded glutaminase that transfers the amido group of glutamine to the large *carB*-encoded subunit. The latter catalyzes the synthesis of CP, a process that involves two phosphorylation reactions and passes through a series of higly unstable intermediates that are shielded from the cytoplasmic environment by transfer from one catalytic site to the next through a 96-Å-long internal tunnel (Holden et al. 1999). The C-terminal part of the large subunit also carries the binding sites for the effector molecules (Rubio et al. 1991; Cervera et al. 1996; Czerwinsky et al. 1995; Delannay et al. 1999; Holden et al. 1999; Thoden et al. 1999a, b). CPS is allosterically activated by ornithine and IMP, and inhibited by UMP. Activation by ornithine is crucial in view of the requirement of CP for the conversion of ornithine into citrulline (Fig. 2). This is especially striking in conditions where arginine becomes limiting and both genetic repression of the entire pathway and feedback inhibition of NAGS are lifted, but the pyrimidine pool is high. In these conditions, activation of CPS by accumulating ornithine overrules its inhibition by UMP (Piérard 1966; Delannay et al. 1999). For a more detailed overview of the CPS-catalyzed reactions, reaction intermediates, enzyme structure, and allosteric regulation of enzyme activity, the reader is referred to a recent review (Charlier et al. 2018). Information on the different types of CPS enzymes and other CP producing and consuming enzymes present in all domains of life is provided in Shi et al. (2018).

Regulation of gene expression

Regulation of arginine biosynthetic genes in *E. coli* is mainly at the level of transcription initiation as demonstrated by hybridization of in vivo synthesized pulse labeled RNA to the separated strands of gene specific DNA probes, in vitro coupled transcription-translation assays, microarrays, and real-time quantitative PCR, but the repression coefficients vary widely (Cunin and Glansdorff 1971; Cunin et al. 1975, 1976; Rogers et al. 1971, 1975; Pannekoek et al. 1975; Krzyzek and Rogers 1976; Sens et al. 1977a, b; Dohi et al. 1978; Lissens et al. 1980; Piérard et al. 1980; Piette et al. 1982a, b, 1984; Roovers et al. 1988; Caldara et al. 2006). No evidence for an attenuation type of control as operative in many other aa biosynthetic pathways has been found in the control of arginine biosynthesis, and several hypotheses have been proposed to explain this observation and the potential inadequacy of arginine codons to serve as a basis for such a control (Bény et al. 1982; Cunin et al. 1983, 1986). For an overview of older work on the correspondence between mRNA and enzyme levels the reader is referred to Cunin et al. (1986) and Charlier and Glansdorff (2004).

Arginine biosynthetic genes are scattered all over the chromosome in *E. coli* and *S. typhimurium*, except for the

argECBH and *carAB* operons (Glansdorff 1965; Cunin et al. 1969; Mergeay et al. 1974; Gigot et al. 1980; Kilstrup et al. 1988), as later confirmed by whole-genome sequencing. The *argECBH* cluster is organized as a bipolar operon in which the *argE* and *argCBH* wings are transcribed from a pair of facing promoters that share a central operator site (Fig. 3) (Cunin et al. 1969; Elseviers et al. 1972; Jacoby 1972; Pouwels et al. 1974; Panchal et al. 1974; Bretcher and Baumberg 1976; Boyen et al. 1978; Charlier et al. 1978, 1979, 1992; Piette et al. 1982a). This organization results in the production of mRNA molecules with a short (13 nt) overlap at their 5′-end. Whether this complementarity has any regulatory function remains an open question. A weak constitutive secondary promoter for *argE* transcription is located within the *argC* gene. The *carA* and *carB* genes form an operon, polarized from *carA* to *carB*, that is transcribed from a pair of tandem promoters in *E. coli* and *S. typhimurium* (Piette et al. 1984; Bouvier et al. 1984; Lu et al. 1992).

Transcription of all arginine biosynthetic genes is subjected to arginine-specific repression by the arginine repressor ArgR, as first discovered for end-product repression by Vogel (1957, 1961), Maas (1957, 1961), and Gorini et al. (1961). The term repression was first coined in this context by Vogel (1957), as a regulatory mechanism opposed to induction of the *lac* operon. For a historical overview of the development and evolution of early ideas/concepts of bacterial gene regulation, and the importance of studies on arginine metabolism in this process see Maas (1991, 2007). The *argR* gene itself is transcribed from two promoters, a weak constitutive upstream promoter, and a negatively autoregulated one, 75 bps more downstream (Lim et al. 1987, 1988). ArgR was estimated to be present at about 500 molecules per cell in minimal medium and 300 in arginine-supplemented medium (Tian et al. 1994), which is in the upper range of concentrations of the vast majority of bacterial TFs other than NAPs (nucleoid-associated proteins) for which the concentration has been determined (Ishihama et al. 2014). As the arginine biosynthetic genes are scattered, but all subjected to repression by ArgR, they are part of the ArgR regulon, a term first coined by Maas and Clark (1964) to describe this organization, even though they exhibit widely varying repression coefficients (Caldara et al. 2006) and degrees of in vitro and in vivo ArgR binding (Charlier et al. 1992; Cho et al. 2015). As further developed below, the ArgR regulon also comprises the own gene (negatively autoregulated), the *carAB* operon, various genes and operons of arginine transport and catabolism, and many others, as recently demonstrated by high-throughput sequencing of chromatin immunoprecipitated DNA (ChIP-exo) and genome-wide expression profiling (Cho et al. 2011, 2015). Noteworthy, ArgR (alias XerA) also fulfills another function in site-specific DNA recombination, where it is an essential accessory element involved in the generation of the synaptic complex that dictates the direction of the XerCD catalyzed site-specific recombination reaction at the *cer* site, a reaction that resolves multimers of ColE1-type plasmids into the monomeric constituents (Stirling et al. 1988). Remarkably, in this process ArgR acts in concert with PepA (Aminopeptidase A, alias XerB) to impose the directionality of the recombination reaction, and both ArgR and PepA are equally involved in transcriptional control of the *carAB* operon (Stirling et al. 1989; Charlier et al. 1995).

After initial partial purifications of ArgR (Udaka 1970; Kelker et al. 1976), cloning, sequencing, and overexpression

Fig. 3 Sequences of the promoter/operator regions of arginine biosynthetic and transport (import) genes that are subjected to ArgR-mediated transcriptional repression with indication of promoter elements (gray-colored letters) and ArgR-binding sites (ARG boxes, shaded background)

allowed the preparation of highly pure ArgR from *E. coli* K-12 (Eckhardt 1980; Lim et al. 1987). This paved the way for its biochemical and structural characterization, and in vitro ArgR-operator binding and transcription assays. In solution, *E. coli* ArgR is a 98 kDa homohexamer, organized as a dimer of trimers. The structure of the monomeric basic N-terminal DNA-binding domain was solved by NMR spectroscopy (Grandori et al. 1995; Sunnerhagen et al. 1997) and shown to belong to the winged helix-turn-helix (wHTH) family (Brennan 1993). Bacterial ArgR proteins appear to be the sole representatives of one of the 54 families in which bacterial TFs have been divided (Ishihama 2010). The residues Gln38, Ser42, and Arg43, which are exposed on the solvent part of the recognition helix, were shown to be crucial for DNA binding by mutant studies, and are highly conserved among ArgR proteins of other bacteria (Tian and Maas 1994; Miltcheva Karaivanova et al. 1999; Ni et al. 1999). The structure of the unliganded and arginine-bound acidic C-terminal domain of *E. coli* ArgR was solved by X-ray crystallography and shown to consist of a dimer of trimers consolidated by six arginine molecules that bind at the trimer–trimer interface (Van Duyne et al. 1996). Nanoelectrospray ionization time-of-flight mass spectrometry confirmed the hexameric state of both apo- and holo-ArgR (Samalíková et al. 2005). Quantitative analyses of subunit assembly, affinity, stoichiometry, and arginine and DNA sequence specificity of the binding indicated that subunit assembly does not play a role in activation of the DNA-binding capacity but suggest that communication among subunits is required for binding specificity, and that DNA is also an allosteric effector (Szwajkajzer et al. 2001). In the crystal structure, each arginine ligand makes ten hydrogen bonds with the protein (Van Duyne et al. 1996). Seven of these are established between the amino and carboxylate groups and both main-chain and side chains atoms of two subunits belonging to one trimer and one between the $N^{\eta 2}$ atom of arginine and the amide side chain of a glutamine residue of the same trimer. In addition, the guanidino group makes a pair of hydrogen bonds with a conserved aspartate residue of the opposing trimer. Based on this knowledge of the arginine binding pocket, a superrepressor with predicted altered binding specificity for citrulline rather than arginine was constructed by substituting Asp128 by Asn (Niersbach et al. 1998). Effector binding specificity was also confirmed by computational methods (Kueh et al. 2003; Asi et al. 2003). Isothermal titration calorimetry (ITC) and surface plasmon resonance studies of arginine binding revealed that binding of a single effector molecule to hexameric ArgR triggers a global conformational change that reduces the affinity of the remaining five sites by approximately 100-fold, thus resulting in an anti-cooperative effect that potentially introduces a buffering capacity in the regulatory system (Jin et al. 2005; Strawn et al. 2010). However, it is presently not known how the effect of arginine binding to the C-terminal domain is propagated through the molecule to enhance the DNA-binding affinity and specificity of the N-terminal domain of holo-ArgR, and how many arginine molecules are required per hexamer to generate this effect. There is presently no structure available of *E. coli* ArgR holorepressor or DNA-bound ArgR. In contrast, the structure of full-length hexameric apo-ArgR and C-terminal arginine bound oligomerization domain of *G. stearothermophilus* ArgR has been determined (Ni et al. 1999). On the basis of these structures, a model was presented in which arginine binding induces a rotation by 15° of one trimer with respect to the opposite one. Modeling indicates that this would allow the docking of the recognition helices of two pairs of subunits (each pair consisting of subunits belonging to opposite trimers) into four major groove segments aligned on one face of the bend operator DNA helix. However, whether a similar arginine-induced conformational change would occur in *E. coli* ArgR is not proven, and even unlikely, in view of the different oligomerization contact between the two repressors (Ni et al. 1999). Further information on arginine repressor structures of *B. subtilis* (AhrC) and *Mycobacterium tuberculosis* and their complexes with DNA is provided in (Glykos et al. 1998, Garnett et al. 2008; Cherney et al. 2008, 2009).

Enzymatic and chemical footprinting demonstrated that hexameric fully saturated arginine-bound *E. coli* ArgR contacts four helical turns comprising two conserved but slightly different ARG boxes consisting of 18 bp imperfect palindromes separated by three bps (two in *argR*) in each operator and makes contacts with major and minor groove determinants, all aligned on one face of the DNA helix (Figs. 3, 4) (Charlier et al. 1992; Tian et al. 1992; Lu et al. 1992; Wang et al. 1998). Remarkably, the 3 bp spacer connecting two ARG boxes invariably consists exclusively of A–T and T–A bps. Similarly, the center of each ARG box is also very A + T rich (Fig. 4). ArgR binding to a pair of ARG boxes induces DNA bending by approximately 70°–90° as determined for the *argF* operator (Tian et al. 1992; Burke et al. 1994), and 99° for the *carAB* operator (Devroede 2006). The local A + T rich linker sequences connecting major groove segments contacted by ArgR must facilitate local minor groove compression that accompanies DNA bending, as underscored by the difference in binding affinity of G–C and I–C substitution mutations of A–T pairs in these regions (Wang et al. 1998). An I–C pair is identical to G–C in the major groove and to an A–T pair in the minor groove but inosine lacks the exocyclic NH_2 group of guanine that protrudes in the minor groove and inhibits groove compression. Premodification binding interference studies performed with the operators of the *carAB* and arginine transport genes (*artJ*, *artP*, and *hisJ*, see below) and saturation mutagenesis revealed the most crucial positions for complex formation and the importance of minor groove

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
argA	A	C	A	G	A	A	T	A	A	A	A	A	T	A	C	A	C	T
argA	T	T	C	G	A	A	T	A	A	T	C	A	T	G	C	A	A	A
argE-CBH	T	A	T	C	A	A	T	A	T	T	C	A	T	G	C	A	G	T
argE-CBH	T	A	T	G	A	A	T	A	A	A	A	A	T	A	C	A	C	T
argD	A	G	T	G	A	T	T	T	T	T	T	A	T	G	C	A	T	A
argD	T	G	T	G	G	T	T	A	T	A	A	T	T	T	C	A	C	A
argF	A	A	T	G	A	A	T	A	A	T	T	A	C	A	C	A	T	A
argF	A	G	T	G	A	A	T	T	T	T	A	A	T	T	C	A	A	T
argG	A	T	T	A	A	A	T	G	A	A	A	A	C	T	C	A	T	T
argG	T	T	T	G	C	A	T	A	A	A	A	A	T	T	C	A	G	T
argG	T	G	T	G	A	A	T	G	A	A	T	A	T	C	C	A	G	T
argI	A	A	T	G	A	A	T	A	A	T	C	A	T	C	C	A	T	A
argI	A	T	T	G	A	A	T	T	T	T	A	A	T	T	C	A	T	T
carAB	T	G	T	G	A	A	T	T	A	A	T	A	T	G	C	A	A	A
carAB	A	G	T	G	A	G	T	G	A	A	T	A	T	T	C	T	C	T
artJ	T	T	A	A	A	T	T	A	T	T	T	A	A	T	C	A	T	G
artJ	A	T	T	G	C	A	T	A	T	A	A	A	T	T	C	A	A	T
artP	T	T	T	A	A	C	T	T	T	T	A	A	A	G	C	A	G	A
artP	A	T	T	G	C	A	T	A	A	T	T	A	T	T	C	T	G	T
hisJ	A	A	T	G	C	A	T	A	C	T	T	A	A	G	C	A	T	T
hisJ	A	A	T	G	A	A	A	A	A	T	A	A	T	A	C	G	T	C
argR	T	T	T	G	C	A	T	A	A	A	A	A	T	T	C	A	T	C
argR	T	A	T	G	C	A	C	A	A	T	A	A	T	G	T	T	G	T
A	12	7	2	3	16	18	1	15	14	10	12	22	3	4	0	19	4	7
T	11	9	20	0	0	3	21	5	8	13	8	1	18	10	1	3	9	13
G	0	6	0	19	1	1	0	3	0	0	3	0	0	7	0	1	6	1
C	0	1	1	1	6	1	1	0	1	0	0	0	2	2	22	0	4	2
Consensus	A	N	T	G	A	A	T	A	A	T	A	A	T	N	C	A	N	T
	T							T	T	A	T							A
			M						m			M						

Fig. 4 Alignment of ARG box sequences present in the control regions of the ArgR repressible arginine biosynthetic and transport (import) genes of *E. coli* with indication of frequencies of occurrence of the various nucleotides at every position, and deduced consensus sequence. M and m refer to Major and minor groove segments of the DNA helix contacted by ArgR (Wang et al. 1998)

compressibility at the center of the operator (Wang et al. 1998; Caldara et al. 2007). In vivo repressibility and in vitro binding affinity showed good correlation (Charlier et al. 1992), in agreement with the previous observation that regulation of gene expression in the arginine biosynthetic pathway is essentially at the transcriptional level. It appears that ArgR-mediated repression of arginine biosynthetic genes prevails during metabolic steady state growth conditions, whereas growth rate linked global regulation determines the maximal biosynthetic promoter activities during transition periods from one steady state to another (Gerosa et al. 2013). In the divergently transcribed *argECBH* operon, a single pair of ARG boxes constitutes the ArgR-binding site that controls the expression of both wings of the bipolar operon (Fig. 3) (Charlier et al. 1992). Stoichiometry experiments demonstrated the binding of one hexamer per pair of ARG boxes in the *argECBH*, *carAB*, and *argF* operators, suggesting that each Arg box is contacted by two subunits of the hexameric assembly, leaving another two subunits unbound (Charlier et al. 1992; Tian et al. 1992). Interestingly, the *argG* operator bears a pair of ARG boxes overlapping the promoter, and an additional single upstream ARG box (Fig. 4) that is contacted by the same hexamer, resulting in outlooping of the intervening DNA (Charlier et al. 1992). This single ARG box is located slightly upstream of the −35 promoter region of the divergently transcribed *metY* gene that encodes one of two methionine initiator tRNAs (Krin et al. 2003). Furthermore, the intergenic *metY–argG* region (startpoint of transcription 187 nt apart) bears a binding site for CRP (cyclic-AMP receptor protein) located between the single ARG box and the *metY* promoter, and from this position the cAMP-CRP complex activates *argG* expression (about 2-fold), but inhibits *metY* transcription (about 16-fold) and ArgR interferes with this inhibition, thus generating a link between arginine metabolism and translation initiation (Krin et al. 2003). *Escherichia coli* but not *S. typhimurium argD* was identified by microarray experiments as a member of the PhoPQ regulon, a two-component system that responds to Mg^{2+} starvation, and the *argD* control region bears a PhoP box that overlaps the transcription start and downstream ARG box (Fig. 3) (Monsieurs et al. 2005) and *argA* is also a target for Lrp (leucine responsive regulatory protein) (Cho et al. 2011).

In all arginine biosynthetic operators except *argA*, the downstream promoter P2 of the *carAB* operon, and the downstream ArgR repressible promoter of *argR*, the

ArgR-binding site partially overlaps the −10 promoter element and transcription start site(s), though to a variable degree (Charlier et al. 1992; Tian et al. 1992), whereas the ARG boxes in the operators of arginine import genes are located slightly more upstream (Fig. 4; see alo below: Arginine transport) (Caldara et al. 2007). In vitro binding of ArgR and RNAP to the bipolar *argECBH* control region and the *car*P2 promoter was shown to be mutually exclusive (Piette et al. 1982a; Charlier et al. 1988). In contrast, ArgR bound to the *car*P2 promoter appears to be unable to arrest transcription initiated 67 nt more upstream at the pyrimidine-specific promoter *car*P1 (Charlier et al. 1988). The latter is subjected to multiple controls, including stringent control by the alarmone ppGpp, UTP-dependent stuttering, and transcriptional activation and repression by multiple DNA-binding transcription factors (TFs), nucleoid-associated factors, and trigger enzymes (*bona fide* enzymes with an unrelated regulatory function in gene expression; Commichau and Stühlke 2008), including RutR, PurR, IHF (integration host factor), PepA (aminopeptidase A), and PyrH (UMP-kinase), and effector molecules originating from pyrimidine and purine biosynthesis (for a recent overview of *carAB* regulation see Charlier et al. 2018).

As developed above, in *E. coli* K-12 and *E. coli* W, arginine acts as a co-repressor for ArgR and enhances its binding affinity and sequence specificity. This results in partial repression due to endogenously produced arginine upon growth on minimal medium and enhanced repression upon arginine supplementation of the medium. However, a different pattern is observed in *E. coli* B, where arginine biosynthetic genes are repressed on minimal medium but slightly derepressed upon arginine supplementation (Gorini and Gundersen 1961). This difference in behavior between *E. coli* K-12 and *E. coli* B could be attributed to a single aa substitution in the *argR* gene (leucine instead of proline at position 70) (Lim et al. 1988; Tian et al. 1994). In vitro binding assays demonstrated that the arginine-bound K-12 repressor ($ArgR_K$) has a higher affinity for the *argF* operator than the $ArgR_B$ homolog, whereas the opposite holds for the unliganded proteins. This stronger binding of apo-$ArgR_B$ explains the repression observed in *E. coli* B grown under arginine-limiting conditions. The slight derepression observed upon arginine supplementation results from a reduced affinity of the arginine-bound form and a lower concentration of $ArgR_B$ due to enhanced autorepression (Tian et al. 1994). Hence, $ArgR_B$ acts as a superrepressor in the absence of arginine supplementation as further demonstrated by the isolation of various superrepressor mutants of *E. coli* K-12, among which a proline to leucine substitution at position 70 (Tian and Maas 1994). These results suggest that *E. coli* B is a superrepressor mutant derived from *E. coli* K-12, a view that is supported by the observation that the $argR_B$ allele is rare among isolates from natural sources (Merlo et al. 2006). Competition experiments indicate that the $argR_K$ and $argR_B$ alleles reflect adaptations to different natural habitats (Suiter et al. 2003). The $argR_K$ type was selectively favored upon growth in arginine-supplemented medium and disfavored in minimal medium. Furthermore, whereas short cycling rates between low and high arginine concentrations favored the $argR_K$ type, long cycles favored the $argR_B$ type, suggesting that weak constitutive expression as observed in *E. coli* B, may in specific circumstances be an adaptative strategy that constitutes a selective advantage over strong regulation.

Arginine catabolism

Different microorganisms use distinct routes for the catabolism of arginine that, again dependent on the organism and the growth conditions, may serve as a sole source of nitrogen, carbon and energy (Cunin et al. 1986; Reitzer 2005; Lu 2006). *Escherichia coli* and *S. typhimurium* use essentially the arginine succinyltransferase (AST) pathway (Fig. 5) for degradation and use of arginine as sole source of nitrogen in nitrogen-limiting conditions (Schneider et al. 1998; Lu and Abdelal 1999). In these growth conditions, arginine decarboxylation by the ADC pathway does not contribute much to arginine consumption (about 3%) (Schneider et al. 1998). In contrast, arginine decarboxylation is important for the biosynthesis of polyamines in ammonia-containing media (Fig. 6; see below). The AST pathway was first discovered in *Pseudomonas* species where it serves a different function, namely the use of arginine as a source of carbon under aerobic growth conditions, what *E. coli* cannot (Vander Wauven and Stalon 1985; Stalon et al. 1987; Itoh 1997). Genes of the *E. coli* AST pathway are organized in the *astCADBE* operon (Schneider et al. 1998). Interestingly, all the ORFs of the multicistronic operon overlap slightly, which is suggestive of translational coupling (Schneider et al. 1998). Mutations in any of the *ast* genes completely abolish the utilization of arginine as a sole source of nitrogen. Furthermore, a deficiency in *astC* (previously named *argM*) encoding succinylornithine transaminase, but not in any other *ast* gene, also results in strongly impaired ornithine catabolism (Schneider et al. 1998). The *E. coli astCADBE* operon is transcribed from $E\sigma^{54}$ (active during nitrogen limitation in exponential phase) and $E\sigma^{S}$ (active during stationary phase in ammonia-containing medium) promoters. Transcription initiation from these overlapping promoters was shown to be competitive and mutually exclusive (Kiupakis and Reitzer 2002). Expression of the *E. coli ast* operon is subjected to catabolite repression, activated by the transcriptional regulator NtrC under nitrogen-limiting conditions, and superinduced by arginine in an ArgR-dependent manner (Schneider et al. 1998; Fraley et al. 1998; Kiupakis and Reitzer 2002). The latter appears to play an accessory role in the

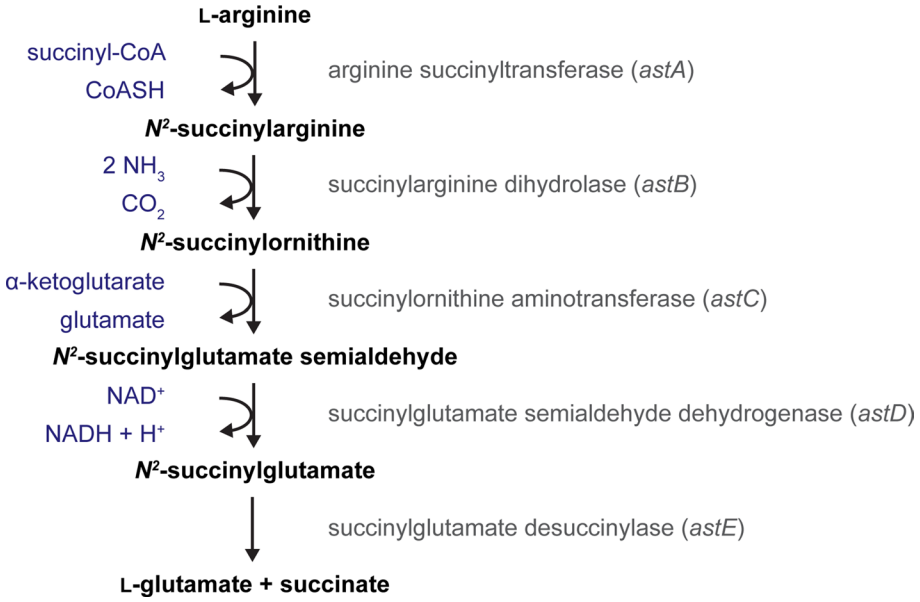

Fig. 5 Arginine succinyl transferase (*ast*) pathway for arginine catabolism

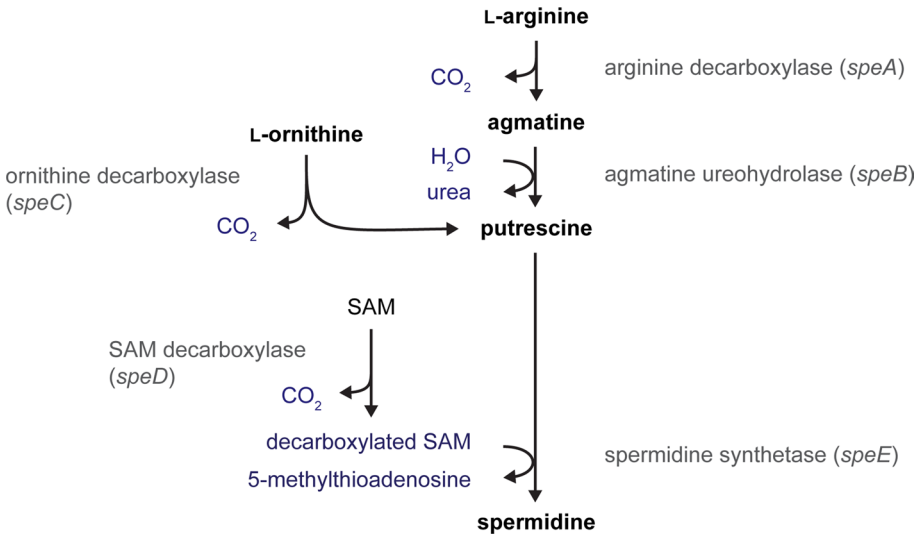

Fig. 6 Biosynthesis of the major polyamines putrescine and spermidine starting from L-arginine and L-ornithine

stimulation of $E\sigma^{54}$ and NtrC-dependent transcription, which is different in *S. typhimurium* (see below). In silico analysis of the *ast* control region revealed the existence of a single potential CRP and two potential NtrC-binding sites in a region extending between 200 and 240 bp upstream of the $E\sigma^{54}$ promoter and seven potential ArgR-binding sites distributed all over the control region. Binding of $E\sigma^{S}$ and $E\sigma^{54}$, NtrC, and ArgR was demonstrated in vitro (Kiupakis and Reitzer 2002). Binding of ArgR to four binding sites was proposed to induce DNA bending and to facilitate the interaction between upstream bound NtrC and $E\sigma^{54}$ (Kiupakis and Reitzer 2002). IHF that induces pronounced DNA bending and frequently acts in conjunction with activators of $E\sigma^{54}$-dependent promoters does not affect *ast* expression in *E. coli*. A similar though different regulatory pattern with a more prominent role for ArgR was proposed for the *astCABDE* operon of *S. typhimurium*. Here, arginine-dependent induction of the operon in nitrogen-limiting conditions (not only superinduction as in *E. coli*) was shown to be abolished in an *argR* mutant and both ArgR and CRP were found to be required for induction of the *ast* operon under carbon starvation (Lu and Abdelal 1999). Binding sites for CRP, NtrC, IHF and several ARG box sequences as potential binding sites for ArgR were identified in the *S. typhimurium ast* control region, and in vitro binding of CRP, ArgR and NtrC to these sites was confirmed by electrophoretic mobility shift assays (EMSAs) and DNase I footprinting (Lu and Abdelal 1999). Transcription of the *E. coli ast* operon is also induced in stationary growth and important for the survival of *E. coli* under conditions of carbon starvation. This explains why *E.*

coli astC has also been designed *cstC*, originally characterized as a starvation gene that exhibits 60% aa sequence identity, 91% similarity with *argD* and was found to be identical to *astC* (Blum et al. 1990; Fraley et al. 1998).

In *E. coli*, arginine is also a substrate for two arginine decarboxylases, SpeA involved in the synthesis of the major polyamines putrescine and spermidine, and AdiA involved in arginine-dependent acid resistance, also called biosynthetic and biodegradative arginine decarboxylase, respectively (Figs. 1, 6) (Morris and Boeker 1983). Decarboxylation of arginine and ornithine is a major pathway for the consumption of arginine and its precursor ornithine as substrates for the synthesis of polyamines in ammonia-containing media (Fig. 6). SpeA is a tetramer of 70 kDa subunits (Wu and Morris 1973). It catalyzes the decarboxylation of arginine into agmatine that is then hydrolysed by agmatine ureohydrolase (SpeB, agmatinase) to form putrescine, which combined with decarboxylated S-adenosylmethionine (SAM) is converted into spermidine by spermidine synthase (SpeE) (Fig. 6). Putrescine may also be directly formed by decarboxylation of ornithine by the biosynthetic ornithine decarboxylase (SpeC) (Morris and Pardee 1965). The latter reaction appears to be predominant in polyamine biosynthesis. SpeA as well as SpeC activity (but not the respective degradative carboxylases AdiA and SpeF) is strongly inhibited at the posttranslational level by a specific antizyme (AtoC) (Canellakis et al. 1993; Panagiotidis et al. 1994, 1995; Hayashi et al. 1996). Antizyme activity was shown to rely on the ribosomal proteins S20 and L34 (Panagiotidis et al. 1995). For more information on the physiological role of polyamines, polyamine biosynthesis and regulation, the reader is referred to Charlier and Glansdorff (2004), Michael (2018) and Igarashi and Kashiwagi (2018).

The importance of arginine and the role of the biodegradative arginine decarboxylase (AdiA) in the survival of *E. coli* in extremely acidic conditions is developed in the next paragraph (Arginine transport, part on *"An arginine:agmatine antiport system for survival in acidic conditions"*).

Arginine transport: uptake, exchange and excretion

ABC-type transporters for arginine and ornithine import

In bacteria, active uptake of amino acids for protein synthesis is often performed by primary transport systems of the ABC-type (ATP-binding cassette) (Hosie and Poole 2001; Saier 2000; Burkovski and Krämer 2002; Davidson and Chen 2004; Biesmans-Oldehinkel et al. 2006; Jung et al. 2006; Davidson et al. 2008; ter Beek et al. 2014; Ford and Beis 2019). Whenever amino acids are required for cellular development and available in the environment, they will be actively pumped in since import is energetically less costly than de novo synthesis. *E. coli* has three such ABC-type transport systems for the import of arginine (Fig. 1) (Rosen 1971; Celis et al. 1973; Higgins and Ames 1981; Wissenbach et al. 1995). In *E. coli* and *S. typhimurium*, they are encoded by two gene clusters: *artPIQM-artJ* (*art* for *ar*ginine *t*ransport) and *argT-hisJQMP*. Both clusters contain two transcriptional units, one monocistronic and one polycistronic, of the same polarity. The three transport systems differ in affinity for arginine binding, substrate specificity, and regulation at the level of gene expression. Combined these characteristics indicate that they serve different functions and operate under different growth conditions. ArtJ, ArtI, ArgT and HisJ are the four periplasmic binding proteins encoded by the gene clusters. ArtJ (ArgBP-I) binds arginine with high affinity (K_d 0.4 µM) but not ornithine (Rosen 1971; Celis et al. 1973; Wissenbach et al. 1995). ArtI appears to be the arginine and ornithine-binding protein (AO) (Wissenbach et al. 1993, 1995; Reitzer and Schneider 2001) instead of the *abpS* gene product, as previously suggested (Celis 1981, 1982). ArgT (LAO) binds the basic amino acids lysine, arginine (K_d 1.5 µM) and its precursor ornithine, and HisJ binds histidine (K_d 0.11 µm) and arginine, though with a much lower affinity (K_d 10 µM) (Kustu and Ames 1973). The $HisQMP_2$ complex is best characterized in *S. typhimurium* and consists of two integral inner membrane proteins, HisQ and HisM that form a translocation pore and associate with two ATP-binding HisP subunits, which provide the energy for active transport (Kerppola et al. 1991; Ames et al. 2001; Heuveling et al. 2014). The periplasmic binding proteins HisJ and ArgT that share 70% aa sequence identity associate with this complex to deliver the substrate(s). An analogous $ArtQMP_2$ complex is supposed to assemble and interact with the periplasmic binding proteins ArtJ and ArtI (Fig. 1).

Transcription initiation at an $E\sigma^{70}$-dependent promoter in the *artJ*, *artPIQM* and *hisJQMP* control region was shown to be repressed by ArgR, though to various extents, with a repression coefficient that is about ninefold higher for *artJ* than for *artP* and *hisJ* (Caldara et al. 2006, 2007). Activated ArgR binds to two similar but non-identical ARG boxes that overlap the −35 promoter element of *artJ* (P_{artJ}) and *artP* (P_{artP}) to the same extent, but binds slightly upstream of the −35 promoter element of *hisJ* (P_{hisJ}) (Figs. 3, 4) (Caldara et al. 2007). Hence, the position of ARG boxes in the control region of transport genes is in all instances different from their location in the control region of arginine biosynthetic genes, where they overlap the −10 promoter element and transcription start site (Fig. 3) (Charlier et al. 1992; Tian et al. 1992; Wang et al. 1998). DNase I footprinting and single-round in vitro transcription assays with ArgR and RNAP indicated that repression exerted by ArgR at P_{artJ} but not at P_{hisJ} is by mutual steric inhibition of binding (Caldara et al. 2007). At P_{hisJ} binding of ArgR prior to the addition

of RNAP also inhibits binding of the latter, but the opposite does not occur and it appears that ArgR can bind to an RNAP-bound P_{hisJ}. On the basis of these results, it was proposed that ArgR-mediated repression of P_{hisJ} does not result from direct inhibition of RNAP binding to overlapping sites, but involves an ArgR-induced conformational change of the promoter that makes it a worse substrate for the polymerase (Caldara et al. 2007). In contrast to all other transcription units of the arginine transport systems, expression of *E. coli argT*, encoding the LAO-binding protein, is not repressed by ArgR and arginine (Caldara et al. 2006). Instead, *argT* transcription relies on $E\sigma^{54}$ and is activated by the nitrogen regulatory protein NtrC in conditions of nitrogen limitation, and downregulated by the alarmone ppGpp (Zimmer et al. 2000).

The *artP* control region was also shown to be a target of Lrp (Leucine-responsive regulatory protein) in a chromatin immunoprecipitation study (Cho et al. 2008, 2011). *E. coli* Lrp is the prototype of a large family of prokaryotic transcriptional regulators also called feast-famine regulators (Calvo and Matthews 1994; Newman and Lin 1995; Brinkman et al. 2003; Kawashima et al. 2008). Lrp frequently regulates gene activity in function of the global energy status of the cell and exerts an additional coordinating control, on top of a more specific regulation. Lrp may either act as a repressor or an activator, and in either case the effect of the regulator may be potentiated (concerted mode) or alleviated (reciprocal mode) by leucine, or be leucine-independent (independent mode) (Cho et al. 2008). In a genome-wide expression profiling study, the expression of the *artPIQM* cluster was found to be approximately sixfold downregulated by Lrp in a leucine-sensitive mode (Hung et al. 2002). Lrp-mediated regulation of P_{artP} is thus according to the reciprocal manner. Work from this laboratory confirmed the effect of Lrp and leucine, and indicates that the binding sites for the two transcription factors partially overlap and that ArgR and Lrp act as competitive repressors in the control of P_{artP} whereby each repressor is more potent in the absence of the other (Torres Montaguth 2014). This interference in the action of ArgR and Lrp may at least in part explain the remarkable difference observed in the ArgR-mediated repressibility of P_{artJ} and P_{artP} (Caldara et al. 2006, 2007) and a higher degree of ArgR-dependent occupancy of the *artJ* operator in vivo (Cho et al. 2015), whereas in contrast, in vitro binding of ArgR to the two control regions occurs with about the same affinity and the overlap of the ArgR binding sites and the respective promoter elements is identical (Caldara et al. 2007) (Fig. 3). Furthermore, it has to be emphasized that still other control mechanisms influence *artP* transcription in stressful conditions and might equally contribute to the observed difference between ArgR repressibility of P_{artJ} and P_{artP}. Thus, a two-fold upregulation of *artP* transcription was observed at the onset of stationary phase growth, and in the presence of paraquat or sodium salicylate (Lacour and Landini 2004). All these observations appear to be linked to the presence of additional $E\sigma^{S}$-responsive promoters in the *artP* control region, upstream of the major $E\sigma^{70}$ promoter, and the action of SdsR, a small regulatory RNA (sRNA) that is transcribed by $E\sigma^{S}$ and accumulates in the stationary phase (Fröhlich et al. 2012). SdsR, in conjunction with Hfq, a sRNA-binding protein that generally acts as an RNA chaperone and favors sRNA–mRNA interactions, was shown to directly affect the expression of the *artPIQM* operon of *S. typhimurium* (Fröhlich et al. 2016). SdsR is highly conserved among *Enterobacteriaceae*, hence a similar mechanism may be operative in *E. coli*.

In view of these findings, it appears that the arginine and arginine plus ornithine specific transport systems encoded by the *artJ-artPIQM* gene cluster are used in exponentially growing cells for the uptake of arginine and its precursor ornithine for protein synthesis, whereas the LAO system encoded by the *argT-hisJQMP* cluster might be used as a scavenging system for basic and nitrogen-rich amino acids as a source of nitrogen in nitrogen-limiting conditions. Consistently, the LAO transport system is not expected to be strongly repressed by arginine. Furthermore, the low ArgR-mediated repressibility of the *hisJQMP* operon might equally be linked to its absolute requirement for histidine transport, as it appears to be the only histidine transport system in *E. coli*, in contrast to *S. typhimurium* that disposes of more than one such system.

An arginine:agmatine antiport system for survival in acidic conditions

E. coli also imports arginine by means of an arginine:agmatine antiport system (Fig. 1) (secondary transport system) that contributes to the remarkable resistance of the organism to very acidic challenges (pH 2 and below) as it faces for instance upon its passage through the stomach. To survive in such conditions, *E. coli* uses an ensemble of sophisticated adaptive strategies in which arginine and glutamate, and to a lower degree ornithine and lysine play an important role. These acid-resistance mechanisms are designed AR1, AR2 (GDAR, glutamate-dependent acid resistance), AR3 (ADAR, arginine-dependent acid resistance), LDAR (lysine-dependent acid resistance) and ODAR (ornithine-dependent acid resistance) (Kanjee and Houry 2013). They are all operative in conditions of stationary phase growth and besides AR1, they all belong to the amino acid-dependent proton-consuming acid-resistance mechanisms that involve the decarboxylation of an amino acid and an antiport system that must assure replenishment of substrate and removal of product of the decarboxylation reaction (Gong et al. 2003; Iyer et al. 2003). AR1 (also called the oxidative system) is acid induced in stationary phase.

Its regulation is poorly understood but AR1 is known to be repressed by glucose, and requires the alternative sigma factor σ^S and CRP (Castanie-Cornet et al. 1999). AR2 is induced upon entry in the stationary phase in an acidic environment (Castanie-Cornet and Foster 2001). It is likely the best studied acid-resistance mechanism and apparently the most robust one, providing the highest degree of protection. It requires external glutamate that is converted into γ-aminobutyric acid (GABA) by two isoforms of glutamate decarboxylase (GadA and GadB) in a process that consumes an intracellular proton and releases CO_2, and GadC, a glutamate:GABA antiporter (Castanie-Cornet and Foster 2001). This process appears to contribute to pH homeostasis (internal pH 4.2 at an external pH of 2.5) and the generation of a proton motive force by draining protons from the cytoplasm, and helps to convert the membrane potential from an inside negative to an inside positive charge. This reversal of $\Delta\Psi$ appears to be more crucial for acid resistance than the maintenance of a specific internal pH (Richard and Foster 2004). Regulation of the GDAR system is complex and involves the AraC-like TFs GadX, GadW and YdeO, GadE (a LuxR-like regulator), PhoP, the two-component regulatory system EvgA/S, the RccCDB phosphorelay, CRP, H-NS (histone-like nucleoid structuring protein, a NAP) and two sigma factors (σ^{70} and σ^S) (De Biase et al. 1999; Castanie-Cornet and Foster 2001; Hommais et al. 2001; Gong et al. 2004; Ma et al. 2002, 2003a, b, 2004; Masuda and Church 2002, 2003; Tramonti et al. 2002; Zwir et al. 2005; Castanie-Cornet et al. 2006). Of these, GadE appears to be the master regulator whereas most other regulators act more indirectly, by affecting the synthesis and activity of GadE (Foster 2004; Sayed et al. 2007; Zhao and Houry 2010).

Most interesting in the context of this review are ADAR (AR3) and ODAR. ADAR acts similarly to GDAR but requires external arginine to protect cells at pH 2.5 (internal pH 4.7) grown in complex medium (Richard and Foster 2004). ADAR relies on the action of the *adiA*-encoded acid-inducible arginine decarboxylase (ADC) that decarboxylates arginine to agmatine using pyridoxal 5′-phosphate as a cofactor (Castanie-Cornet et al. 1999; Lin et al. 1995; Blethen et al. 1968) and the pH-dependent arginine:agmatine antiporter AdiC (YjdE) that belong to the major facilitator superfamily (MFS) of transporters, the largest group of secondary active membrane transporters (Law et al. 2008) (Fig. 1). The optimal pH for induction of decarboxylase activity was determined to be 5.2 but maximal conversion of arginine to agmatine was observed at pH 2.5 (Gong et al. 2003). AdiA mutants were shown to be deficient in arginine-dependent acid resistance, without affecting AR1 and AR2; the enzyme is thus specific for arginine-dependent acid resistance (Castanie-Cornet et al. 1999). Furthermore, *adiC* mutants are deficient in arginine-dependent acid resistance and the exchange of extracellular arginine (arg^+) for intracellular agmatine (agm^{2+}) that normally occurs with a 1/1 stoichiometry (Gong et al. 2003; Iyer et al. 2003; Fang et al. 2007; Tsai and Miller 2013). AdiC, of which the structure was solved, is an integral inner membrane protein with 12 putative transmembrane domains (Fang et al. 2009; Gao et al. 2009, 2010; Kowalczyk et al. 2011; Ilgü et al. 2016) that assembles into a star-shaped decamer (a pentamer of dimers) (Andrell et al. 2009). On basis of the 3D structure AdiC was proposed to cycle between a periplasmic-open and a cytoplasmic-open conformation. These two alternative conformations expose a substrate-binding site to opposite faces of the membrane. Substrate binding would then result in a conformational change and transport passes through the formation of the so called occluded state, in which the substrate is buried in the interior of the transporter (Gao et al. 2010; Kowalczyk et al. 2011). Tyrosine 74 was shown to play a crucial role in the pH-sensing activity of AdiC (Wang et al. 2014). AdiC shows sequence identity with the glutamate:GABA antiporter GadC (22%), the lysine:cadaverine antiporter CadB (35%), and the ornithine:putrescine antiporter PotE (29%), which all belong to the amino acid/polyamine/organocation (APC) superfamily of membrane transporters (Jack et al. 2000; Tomitori et al. 2012). The *adiA* and *adiC* genes are part of the *adi* gene cluster (*adiAYC*) that also comprises *adiY*, encoding a 29 kDa regulatory protein that belongs to the XylS/AraC family of TFs (Stim-Herndon et al. 1996). All three genes are transcribed in the same direction, but whereas Northern blotting indicated that *adiA* and *adiY* are transcribed as a bicistronic mRNA, *adiC* mRNA is monocistronic (Gong et al. 2003). Regulation of the *adi* gene cluster is not fully resolved and appears to be complex. Transcription of *adiA* is strongly induced during anaerobic growth in complex medium and low pH (Auger et al. 1989; Shi et al. 1993) and similarly *adiC* transcription was found to be acid induced (Gong et al. 2003). AdiY is an activator of *adiA*, and overexpression of *adiY* was shown to result in elevated *adiA* expression (Stim-Herndon et al. 1996). Furthermore, maximal *adiA* expression relies on CysB, the regulator of the cysteine biosynthetic pathway (Shi and Bennett 1994) and is differentially affected by the NAPs IHF and H-NS that stimulate and repress *adiA* expression, respectively (Shi et al. 1993; Stim-Herndon et al. 1996; Bi and Zhang 2014).

Similar to ADAR, ODAR uses an acid-inducible decarboxylase (SpeF) to convert ornithine into putrescine with the release of CO_2, and an ornithine:putrescine antiporter (PotE) (Kashiwagi et al. 1991, 1992; Tomitori et al. 2012) (Fig. 1). The ODAR and LDAR systems are proposed to play a role under mild acid stress conditions, as opposed to GDAR and ADAR that are operative at extremely low pH (Kashiwagi et al. 1991). The PotE activity has been studied and a structure/function analysis based on the 3D structure of AdiC allowed the identification of the putrescine-binding

site of PotE (Kashiwagi et al. 1997, 2000; Tomitori et al. 2012). Remarkably, PotE can catalyze both uptake and excretion of putrescine, but whereas uptake is dependent on the membrane potential, excretion requires ornithine for the exchange reaction.

Interestingly, acid-adapted strains of *E. coli* K-12 harbor mutations eliminating at least one aa decarboxylase or downregulating its expression by inactivating a transcriptional regulator (e.g., *adiY*) (He et al. 2017). Such mutations/adaptations reduce futile energy consumption upon growth at constant pH.

Arginine excretion by ArgO

Excretion of arginine and its toxic plant-derived analogue canavanine is performed by *E. coli* ArgO (*arg*inine *o*utward, Fig. 1), as originally identified by Nandineni and Gowrishankar (2004). ArgO mutants are hypersensitive to canavanine, whereas ArgO overproducers allow the cross-feeding of arginine auxotrophs (Nandineni and Gowrishankar 2004). Active export of amino acids was discovered relatively recently and may at first sight appear incongruous. Nevertheless, various export systems for amino acids have been identified (Bröer and Krämer 1991a; Franke et al. 2003; Livshits et al. 2003; Eggeling and Sahm 2003; Kutukova et al. 2005; Doroshenko et al. 2007; Jones et al. 2015). The very first biochemically characterized one was LysE from *C. glutamicum* that exports the basic amino acids lysine and arginine and is considered the paradigm of a superfamily of secondary transmembrane solute translocators that are energized by the proton motive force (Bröer and Krämer 1991a, b; Vrljic et al. 1996, 1999). They might serve as security valves when the intracellular aa concentration becomes too high and toxic due to high uptake and limited catabolic capacity of the cell or as the result of an imbalanced metabolic overflow (Krämer 1994; Aleshin et al. 1999; Burkovski and Krämer 2002), generates harmful osmotic effects (Danchin 2009; Nandineni et al. 2004), or creates an imbalance in the ratios of different amino acids. The latter appears to be the case for ArgO, which is regulated in function of the intracellular concentrations of arginine and lysine and is only activated by the combination of an elevated arginine and concomitant low lysine pool (Nandineni and Gowrishankar 2004; see also below). The importance of such exporters is underscored by the observation that mutants deficient in aa export systems are hypersensitive to a high extracellular aa concentration, whereas inversely, overexpression of the exporter results in enhanced resistance to high intracellular concentrations (Doroshenko et al. 2007).

Interestingly, *E. coli* ArgO (YggA) exhibits sequence similarity (35% identity, 50% similarity) with its ortholog LysE from *C. glutamicum,* and LysE can functionally substitute for ArgO. Nevertheless, the two proteins possess a distinct membrane topology, especially in the N-terminal part, and ArgO does not efficiently excrete lysine (Pathania et al. 2016). In *E. coli*, lysine is excreted by a distinct membrane protein, LysO, that shows 35% aa sequence identity and 50% similarity with ArgO (Pathania and Sardesai 2015). LysE from *C. glutamicum* is controlled at the transcriptional level by LysG (Bellman et al. 2001). Similarly, *argO* transcription is regulated by ArgP and not by ArgR (Nandineni and Gowrishankar 2004; Peeters et al. 2009). LysG and ArgP exhibit significant sequence similarity and are members of the large family of bacterial LysR-type transcriptional regulators (LTTR) with 46 members in *E. coli* (Maddocks and Oyston 2008; Tatusov et al. 2000; Ishihama 2010). However, the activity of the two TFs is differently affected by lysine that counteracts the activating effect of ArgP but stimulates transcription initiation by LysG. Marbaniang and Gowrishankar (2012) demonstrated that LysG from *C. glutamicum* is able to interact with the *E. coli* RNA polymerase to activate its cognate *lysE* target, but neither ArgP nor LysG can regulate the expression of the non-orthologous target gene. However, some single amino acid ArgP substitution mutants were able to activate the orthologous *lysE* indicating some cross-regulation between LysG and some ArgP mutants. *E. coli* ArgP is a dimeric protein of approximately 33.5 kDa subunits in solution but stoichiometry experiments indicate that it forms a tetramer on DNA (Nguyen Le Minh et al. 2018). The intracellular ArgP concentration was estimated to be approximately 0.2–0.8 µM (50–200 dimers/cell) (Hwang and Kornberg 1992; Ali Azam et al. 1999) and the regulator is one of the 12 most abundant nucleoid-associated proteins (NAPs) involved in chromosome structuring (Azam and Ishihama 1999). The 3D structure of *E. coli* ArgP is not known, but the structure of its homolog from *Mycobacterium tuberculosis* (ArgP, alias $LysG^{Mt}$) was solved and revealed the presence of two subunits with a different conformation in the assymetric unit, one open and one closed, and the existence of two forms of dimer assembly, a DNA-binding type and a regulatory domain type (Zhou et al. 2010). $LysG^{Mt}$ exerts autoregulation and positively upregulates the expression of the neighboring gene (that shows sequence similarity with *lysE* from *C. glutamicum*) in the presence of lysine by binding to an inverted repeat in the intergenic region of the divergently transcribed genes that are initiated from overlapping promoters (Schneefeld et al. 2017). There is presently no structure of an effector or DNA-bound ArgP that could provide further insight in the precise mode of action of ArgP. Originally, *E. coli* ArgP (alias IciA for *I*nhibitor ori*C* *i*nitiation) was identified as a protein that binds the origin of DNA replication (*oriC*), inhibits replication initiation at *oriC* in vitro, and stimulates transcription of *dnaA* and *ndr*, both involved in DNA replication (Hwang and Kornberg 1990; Hwang et al. 1992; Lee et al. 1997; Han et al. 1998). However, as *iciA* (*argP*) mutants do not show

any significant replication-associated phenotype, the physiological role of ArgP in DNA replication remains vague. In contrast, ArgP was clearly shown to bind arginine and lysine and to activate *argO* expression in an arginine-dependent manner by binding to an approximately 50–55-bp-long target site in the *argO* control region (Nandineni and Gowrishankar 2004; Laishram and Gowrishankar 2007; Nguyen Le Minh et al. 2018). Effector-free ArgP was equally shown to bind to and activate transcription of several other genes involved in aa metabolism and transport, of which *dapB* (dihydropicolinate reductase), *gdhA* (glutamate dehydrogenase), and *lysP* (a lysine-specific transporter of the APC family) are the best characterized examples (Bouvier et al. 2008; Goss 2008; Ruiz et al. 2011; Marbaniang and Gowrishankar 2011, Nguyen Le Minh et al. 2018). ArgP thus activates the excretion of arginine (ArgO) and the uptake of lysine (LysP), but whereas activation of *argO* is arginine-dependent and lysine-sensitive, stimulation of *lysP* transcription does not require arginine but is equally lysine-sensitive (Laishram and Gowrishankar 2007; Nguyen Le Minh et al. 2018). Remarkably, both *argO* and *lysP* are also controlled by Lrp (Peeters et al. 2009; Ruiz et al. 2011). Lrp-mediated activation of *argO* is leucine-sensitive (reciprocal mode) and interferes with ArgP-mediated activation due to steric overlap of their respective binding sites (Peeters et al. 2009). Hence, ArgP and Lrp act as competitive activators of *argO* transcription.

Arginine and lysine compete for binding to a unique or overlapping effector binding site(s) on ArgP (Laishram and Gowrishankar 2007). ArgO is the only ArgP target that requires arginine-bound ArgP (ArgP-arg) for activation. All other investigated ArgP targets are activated by effector-free ArgP and lysine counteracts this effect, mainly by reducing the DNA-binding affinity (Bouvier et al. 2008; Laishram and Gowrishankar 2007; Marbaniang and Gowrishankar 2011; Nguyen Le Minh et al. 2018). In this context, it is worth noticing that the ArgP-binding site of *argO* overlaps the −35 promoter element, whereas it is located slightly more upstream in the control region of all other characterized target genes where it is evidently positioned correctly to allow the establishment of apo-ArgP-RNAP contacts (Nguyen Le Minh et al. 2018). Binding of ArgP to the *argO* control region does not require arginine and is not counteracted by lysine, but only ArgP-arg stimulates transcription. Laishram and Gowrishankar (2007) elegantly demonstrated that both ArgP-arg and ArgP-lys (but not apo-ArgP) stimulate the recruitment of RNAP and open complex formation. However, whereas arginine stimulates productive transcription, ArgP-lys restrains RNAP at the promoter and traps it in an unproductive complex. Mutant studies and high-resolution contact probing experiments revealed that the activating (ArgP-arg) and repressing (ArgP-lys) forms of the regulator make slightly different contacts with the *argO* operator, whereby one of two ArgP-arg dimers forming a DNA-bound tetramer contacts a different, additional binding site in the *argO* operator that is not present in the other targets (Nguyen Le Minh et al. 2018). Such a mechanism is in agreement with the sliding dimer model proposed for other LTTRs (Maddocks and Oyston 2008).

Conclusions

The study of arginine biosynthesis, catabolism and traffick of the aa over the cytoplasmic membrane has delivered significant contributions to numerous aspects of microbial physiology and revealed unexpected connections with other amino acid metabolic pathways, in particular lysine and leucine, and other, at first sight totally unrelated cellular processes.

From the historical perspective, the occurrence of two OTC encoding genes, *argI* and *argF*, in *E. coli* K-12 constituted one of the very first well-documented examples of horizontal gene transfer, and the *argECBH* operon one the very early examples (together with the biotin operon) of a well-characterized bipolar operon transcribed from a pair of facing promoters, sharing an internal control region. Furthermore, exploiting the *argECBH* cluster allowed the selection and study of gene duplications reactivating silent genes by the formation of tandem and inverted repeats, and provided a potential mechanism for the origin of bipolar operons (Charlier et al. 1979, 1983). It also delivered the first unambiguous demonstration that a transposon (IS*3*) may function as a mobile promoter (Charlier et al. 1982). The study of *E. coli* ArgR provided the very first demonstration that a transcriptional repressor may also be involved in gene activation (Bacon and Vogel 1963; Kiupakis and Reitzer 2002). More recent work revealed that ArgR not only functions as a transcriptional regulator but also exerts a totally unrelated function, as a structural component (in conjunction with PepA) in the formation of a synaptic complex that imposes the directionality of a site-specific DNA recombination reaction (Stirling et al. 1988, 1989). Unraveling the regulation of the tandem pair of promoters directing transcription of the CPS-encoding *carAB* operon revealed moonlighting activities of the *bona fide* enzymes PepA (alias *carP* and *xerB*) and PyrH (UMP-kinase, an essential enzyme in *E. coli*) in transcriptional regulation (Roovers et al. 1988; Charlier et al. 1995; Kholti et al. 1998). They are, therefore, trigger enzymes (Commichau and Stühlke 2008). Interestingly, both PepA and ArgR act as essential accessory proteins in the site-specific resolution reaction of ColE1 multimers and are jointly involved in regulation of the P1 and P2 promoters of the *carAB* operon, respectively. In both processes, PepA-induced DNA wrapping plays a crucial role (Minh et al. 2009; Nguyen Le Minh et al. 2016), thus reinforcing this surprising but interesting connection, also pointing to the functional recruitment of existing enzymes

(PepA, PyrH) or proteins originally involved in the control of DNA replication and partitioning (IciA, alias ArgP and ArgR) for gene regulatory tasks.

The arginine biosynthetic genes are scattered over the *E. coli* chromosome, but all are subjected to transcriptional repression by binding of ArgR to similar but slightly different operator sites, though the repression coefficients vary widely (Caldara et al. 2006). Such an organization may have certain advantages over the classical operon structure, as it allows more flexibility in the adjustment of individual enzyme activities. This flexibility might be an absolute requirement since specific intermediates of arginine biosynthesis are also consumed as substrates in other pathways, and some enzymes of arginine biosynthesis catalyze similar reactions in a distinct pathway. Thus, ornithine is used for polyamine biosynthesis and is an allosteric activator of CPS activity, whereas CP is a precursor common to the de novo synthesis of arginine and pyrimidines. Furthermore, *argD*-encoded *N*-acetylornithine aminotransferase was found to be identical to *N*-succinyl-L,L-diaminopimelate:alpha-ketoglutarate aminotransferase (*dapC*) of lysine biosynthesis (Ledwige and Blanchard 1999). Additional connections between arginine and lysine metabolism in *E. coli* are provided by the observation that acetylornithinase (*argE*) is homologous to succinyldiaminopimelate desuccinylase (*dapE*) (Boyen et al. 1992), and that the transport of the two amino acids over the cytoplasmic membrane is interconnected at both the level of the transport systems, and their mode of regulation. Thus, the periplasmic binding protein ArgT and the $HisQMP_2$ translocator are jointly used for the import of the basic amino acids arginine, ornithine and lysine, and regulation of ArgO-mediated excretion of arginine by the transcriptional regulator ArgP is modulated by arginine and lysine, which exert antagonistic effects. Furthermore, ArgP controls both arginine excretion and lysine biosynthesis (*dapB*) and uptake (*lysP*), and upon overproduction ArgO showed weak lysine-binding capacity (Pathania and Sardesai 2015). Remarkably, both *argO* and *lysP* are equally regulated by Lrp, thus creating an additional common link with leucine metabolism (Peeters et al. 2009; Ruiz et al. 2011). This connection is reinforced by the observation that *argA*, the *artPIQM* operon for arginine and ornithine import, and the *astCADBE* operon of arginine catabolism, are equally subjected to Lrp-mediated control (Cho et al. 2011). Some of the arginine, lysine and leucine biosynthetic genes are also paralogs in other microorganisms, indicating an ancestral interconnection between the biosynthesis of these amino acids (Fondi et al. 2007). Thus, four of the enzymes of lysine biosynthesis via the diaminopimelate pathway (DAP), *ask*, *asd*, *dapC* and *dapE* are evolutionary related to *argB*, *argC*, *argD* and *argE*, respectively. And in organisms such as the bacteria *Thermus thermophilus* and *Deinococcus radiodurans* and the archaea *Pyrococcus*, *Thermoproteus* and *Sulfolobus*, which synthesize lysine via the alternative α-aminoadipate route, the enzymes catalyzing the first four steps of the pathway (LysS, LysT, LysU and homocitrate dehydrogenase) are homologs of enzymes of leucine biosynthesis (LeuA, LeuC, LeuD, LeuB), whereas LysZ, LysY, LysJ, and LysK are homologous to ArgB, ArgC, ArgD, and ArgE (Nishida et al. 1999; Miyazaki et al. 2001; Velasco et al. 2002). Similarly, the paralogs OTC (*argI*, *F*) and ATC (*pyrBI*) constitute another particularly striking example of enzyme recruitment. As both enzymes use CP as a common substrate in the carbamoylation of ornithine and aspartate, respectively, they also reflect another connection, here between the de novo synthesis of arginine and pyrimidines.

Arginine metabolism is thus part of a much larger network of cellular interactions. Unraveling the molecular details of the cognate regulatory mechanisms of enzyme synthesis and enzyme activity is thus particularly important to generate a complete and integrated view of microbial metabolism and physiology. This is not only important from the fundamental viewpoint but also of interest for industrial applications, including the construction of improved microbial arginine overproducing strains, especially since the aa is also an interesting nutraceutical (Ikeda 2003).

Even though different microorganisms use alternative pathways for arginine synthesis such as the cyclic versus the linear pathway, the use of an acetylornithine transcarbamylase as in γ-proteobacterium *Xanthomonas campestris* (Morizono et al. 2006; Shi et al. 2005), a *N*-succinyl-L-ornithine transcarbamylase as in *Bacteroides fragilis* (Shi et al. 2006), or still a fused *argA–argH* enzyme with combined NAGS and argininosuccinase activity as in marine γ-proteobacteria (Xu et al. 2000, 2006), a profound understanding of arginine metabolism and transport of *E. coli*, still the best studied microorganism, remains important as it constitutes an excellent material for evolutionary studies, generates a reference frame, revealed and may continue revealing fascinating cellular connections and moonlighting activities, and may inspire additional investigations in the exploration of enzymatic and regulatory diversity among microorganisms.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Research involving human participants and/or animals This article does not contain any studies with human participants or animals performed by any of the authors.

Informed consent Informed consent was obtained from all individual participants for whom identifying information is included in this article.

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