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REVIEW ARTICLE

Signal transduction and adaptive regulation through bacterial two-component systems: the *Escherichia coli* AtoSC paradigm

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Abstract Adaptive signal transduction within microbial cells involves a multi-faceted regulated phosphotransfer mechanism that comprises structural rearrangements of sensor histidine kinases upon ligand-binding and phosphorylation-induced conformational changes in response regulators of versatile two-component systems (TCS), arisen early in bacterial evolution. In *Escherichia coli*, cross-talk between the AtoS histidine kinase and the AtoC response regulator, forming the AtoSC TCS, through His → Asp phosphotransfer, activates AtoC directly to induce *atoDAEB* operon expression, thus modulating diverse fundamental cellular processes such as short-chain fatty acid catabolism, poly-(R)-3-hydroxybutyrate biosynthesis and chemotaxis. Among the inducers hitherto identified, acetoacetate is the classical activator. The AtoSC TCS functional modulation by polyamines, histamine and Ca^{2+}, as well as the role of AtoC as transcriptional regulator, add new promising perspectives in the physiological significance and potential pharmacological exploitation of this TCS in cell proliferation, bacteria–host interactions, chemotaxis, and adaptation.

Keywords Antizyme · AtoSC two-component system · *Escherichia coli* · Histamine · Poly-(R)-3-hydroxybutyrate · Polyamines

Abbreviations

Az	Antizyme
cPHB	Complexed poly-(R)-3-hydroxybutyrate
HAMP	Linker domain in HKs, adenyl cyclases, methyl-accepting proteins and phosphatases
HK	Histidine kinase
IHF	Integration host factor
LPS	Lipopolysaccharides
ODC	Ornithine decarboxylase
RR	Response regulator
SCFA	Short-chain fatty acid
TCS	Two-component system

Introduction

Cells are capable to sense environmental and microenvironmental changes and respond to various growth conditions and a wide range of stimuli, both intracellular and extracellular, through adaptive and protective processes. Various stressors, such as temperature, pH, light, osmotic changes, nutrient deprivation, and the presence of xenobiotics may trigger alterations in gene expression, cell cycle progression, catalysis, protein–protein interactions, and other modifications in cellular physiology (Palotai et al. 2008). These processes involve evolutionary multi-component endogenous signal transduction mechanisms;

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the heat shock response, and the two-component systems (TCS) being typical examples.

The importance of TCSs is supported by plethora of reports on individual components and numerous related reviews in the literature (West and Stock 2001; Gao et al. 2007; Laub et al. 2007). In most eubacteria, two-component proteins typically constitute ~1% of encoded peptides. In pathogenic bacteria they control the expression of important pathogenetic factors, in addition to regulating basic housekeeping functions. The widespread distribution of two-component signal transduction systems in Bacteria and Achaea reflect their biological value as major sensing and response elements to a wide range of environmental insults that are tuned to respond from within milliseconds to hours (Laub et al. 2007). Although TCSs are probably the most efficient means of adaptation to conventional stressful stimuli encountered by bacteria during their lifespan, the plasticity of some of these sophisticated systems may contribute to strain-specific cellular processes and to the acquisition of distinct features and phenotypes, particularly in pathogens (de Been et al. 2006).

In general, nearly all bacteria widely employ TCS signaling. Eukaryotic organisms, from yeasts to mammals, commonly utilize signaling systems like mitogen-activated protein kinase cascades, growth factor pathways, and receptor tyrosine kinases (Wilkinson and Millar 2000). Thus, contrary to eukaryotic cells that use predominantly Ser/Thr and Tyr protein kinases for their intracellular signaling, most bacteria typically encode at least 20–30 two-component proteins and achieve TCS-mediated signaling by the distantly related His → Asp phosphoryl group transfers that also use ATP as the phosphate donor.

To date, no reports on the presence of TCSs in mammalian cells are available, but lower eukaryotes such as yeasts and plants, as exemplified by *Arabidopsis thaliana* and *Oryza sativa*, make use of His → Asp phosphotransfer, albeit in a limited number of signaling pathways (Santos and Shiozaki 2001; Du et al. 2007; Dortay et al. 2008). In *Saccharomyces cerevisiae*, a TCS variation, the multistep His → Asp → His → Asp phosphorelay, transmits osmotic stress signals to a mitogen-activated protein kinase cascade and an analogous system operates during peroxide stress in *Schizosaccharomyces pombe* (Santos and Shiozaki 2001). Provided that phosphorelay proteins prove to be essential for virulence in the human pathogen *Candida albicans*, they are of value in developing chemotherapeutic agents selectively targeting eukaryotic pathogens.

Many organisms have not been studied in detail yet. The elucidation of mechanisms of action and the coordination of molecular component interactions that contribute to the regulation of these multifactorial networks, in both bacteria and eukaryotes, is a vital ongoing research area. It aims to the understanding and consequently to the therapeutic and biotechnological exploitation of adaptive and protective phenotypes in various organisms.

Common features of bacterial two-component systems

A typical TCS consists of a transmembrane dimeric sensor histidine kinase (HK) and a cytoplasmic cognate response regulator (RR) (Parkinson and Kofoid 1992; Stock et al. 2000; West and Stock 2001; Gao et al. 2007). The involvement of these "dyadic relays" that form complex signaling networks to integrate sophisticated sensory behaviors and information-processing strategies was demonstrated initially in the chemotaxis machinery of *E. coli* during the late 1980s (Kofoid and Parkinson 1988). The cross-talk through phosphoryl transfer in TCSs has been identified using CheA, CheY, NRI, and NRII from *Salmonella typhimurium* and *E. coli*, when the interaction of highly conserved sensors and regulators became evident (Ninfa et al. 1988; Stock et al. 1988, 1989). Since then, several studies demonstrated this interaction both in vitro and in vivo, while genome sequencing revealed that most bacteria possess numerous TCSs, with the number of systems increasing with genome size and complexity of the lifestyle of the organism (Ulrich and Zhulin 2007).

Structure–activity aspects of histidine kinases

The common feature of HKs is the ATP-dependent autophosphorylation at a specific His residue in the kinase core and the subsequent phosphoryl transfer to an Asp residue on a cognate RR, thus modifying its conformation and eventually its biological properties. The majority of HKs are transmembrane, homodimeric proteins. Typically, each monomer consists of a variable extracytoplasmic N-terminal sensing domain and a conserved cytoplasmic region. The latter comprises the dimerization and the C-terminal ATP-binding kinase domains and contains the phosphoacceptor His residue in the H-box as well as four unique signature sequence N-, G1-, F- and G2-boxes (Parkinson and Kofoid 1992; Dutta et al. 1999; Grebe and Stock 1999; West and Stock 2001).

Differences in domain organization led to the recognition of two classes of HKs. Class I HKs, exemplified by the *E. coli* transmembrane osmosensor EnvZ, contain the dimerization His phosphotransfer domain in the conserved transmitter region, whereas class II HKs, represented by the chemotactic regulator CheA, possess the His-containing phosphotransfer domain (Dutta et al. 1999). Both classes contain a catalytic ATP-binding domain and use similar four-helix bundle motifs to relay phosphoryl groups to the RRs regulatory domains, yet there are

differences with respect to four-helix bundle formation and activity (West and Stock 2001). HKs also exist as hybrid proteins containing additional domains, such as ArcB, while some may exhibit phosphatase activity towards their cognate RR as a supplementary means to regulate phosphorylation (Grebe and Stock 1999). Additional classification attempts by numerous criteria denote the versatility of HKs. A cluster analysis of 348 HKs identified 11 different subtypes (Grebe and Stock 1999) and a phylogenetic analysis of 336 HKs in the genomes of 22 Bacteria and 4 Achaea assigned HKs to five major types (Kim and Forst 2001). Of these, types I and II possess orthodox kinase domains and are found predominantly in bacteria and in the archaeon *Archaeoglobus fulgidus*, respectively. Types III and IV possess unorthodox kinase domains, while type V comprises the chemosensor CheA, in which the H-box domain is located at the N terminus of the protein.

Structure–activity aspects of response regulators

Response regulators are the final elements of TCS phosphotransfer before switching to output response regulation and typically function as DNA-binding transcription factors that activate or repress gene transcription, although some may serve as enzymes or as protein–protein interaction domains (Galperin 2006; Gao et al. 2007). In general, RRs are single or multidomain proteins. Their conserved N-terminal regulatory or receiver domain contains the site of phosphorylation and a variable C-terminal effector domain that elicits the output function. Interaction with their partner HKs, phosphotransfer to Asp residues, and phosphorylation-dependent regulation of their effector activities is usually associated with their regulatory domains, which have a $(\beta\alpha)_5$ fold, conformational changes being induced by phosphorylation of the active-site Asp (West and Stock 2001).

The homology of DNA-binding domains of the structurally described effectors classified RRs are into three major subfamilies, the OmpR-PhoB, the NarL-FixJ, and the NtrC-NifA (West and Stock 2001). The largest OmpR-PhoB subfamily accounts for ~44% in *E. coli* and ~30% of all RRs, ~50% of those possessing a DNA-binding domain (Galperin 2006). OmpR is a member of the winged-helix-turn-helix DNA-binding proteins. It regulates the expression of outer membrane porins in enteric bacteria by activating transcription through DNA binding and productive interaction with RNA polymerase (Martínez-Hackert and Stock 1997).

In the second NarL-FixJ subfamily, the structural model nitrate-responsive NarL is composed of the N-terminal regulatory domain containing the Asp residue and the C-terminal output domain comprising the recognition determinants for DNA binding, joined by a 10-residue helix and a 12-residue flexible linker (Maris et al. 2005).

The nitrogen regulatory protein NtrC of the NtrC-NifA subfamily is a three-domain enhancer-binding protein that ultimately activates the transcription of as much as 2% of the bacterial genome (Zimmer et al. 2000). Typically, these RRs are composed of the N-terminal regulatory domain, which in 50% of cases is a TCS receiver domain, the central oligomerization/AAA+ ATPase domain containing the Gly-rich (GXXXXGK) "Walker A" motif and directly involved in transcriptional activation (Flashner et al. 1995) and the C-terminal helix-turn-helix DNA-binding domain recognizing enhancer-like sequences. Upon Asp phosphorylation-mediated activation, the inactive dimeric central ATPase domain oligomerizes and remodels σ^{54} holoenzyme-DNA complexes from the closed to the productive open form using ATP hydrolysis (Pelton et al. 1999). The NtrC N-terminal domain is similar to that of CheY receiver domain controlling flagellar rotation and some constitutively active mutant forms can generate output without being phosphorylated (Bourret and Stock 2002). Integration host factor (IHF) binding sites on the target gene are required for optimal contact of the enhancer-like element-bound NtrC-type transcription factor with the promoter-bound σ^{54} RNA polymerase (Reitzer and Schneider 2001). A conserved GG doublet is significant in σ^{54} holoenzyme complex formation (Barrios et al. 1999). Yet, the structural models proposed for activated NtrC described differences underlying positive and negative regulation for this family (Lee et al. 2003; De Carlo et al. 2006).

While regulation of RR phosphorylation by the accompanying HK has received increased attention, mainly site-directed mutagenesis studies point to the presence of alternate mechanisms involved in RR activation in vivo, including autophosphorylation using small molecules as phosphodonors and autophosphatase activity (West and Stock 2001; Bourret and Stock 2002; Merighi et al. 2003).

Cross-talk between histidine kinases and response regulators

In the simplest cases, the sensor HK and the cognate RR, encoded by the same operon, interact in a one-to-one fashion at the early cross-talk stages of a network committed to downstream regulation. This typical one-to-one cross-talk involves stimulus-regulated HK in *trans* autophosphorylation and subsequent His → Asp phosphotransfer to the cognate RR. The effector domain is thus activated to elicit the specific output response, most commonly transcriptional regulation. Ultimate control of the output response in this most prevalent signaling scheme in prokaryotes is accomplished by the level of RR phosphorylation determined by

RR intrinsic autophosphatase activity and additional influencing factors (Stock et al. 2000; West and Stock 2001). Systematic analysis of activity and specificity of HK autophosphorylation and RR transphosphorylation classified the *E. coli* TCS pairs into quick, medium, and slow, based on the kinetic pattern of HK autophosphorylation in vitro, which may partly correlate with the nature of HKs and RRs such as the need for rapid response to environmental changes (Yamamoto et al. 2005).

One-to-many and many-to-one topologies also exist, thus expanding the basic phosphotransfer pathway to phosphorelays in order to provide effective regulation through increased numbers of loci and complexity. Comparing TCSs to phosphorelays, additional regulator and phosphotransferase domains in the latter and phosphotransfer in the order His → Asp → His → Asp provide more regulation targets in complex pathways used in eukaryotic signaling and in bacterial sporulation or cell cycle control (Hoch 2000; Laub et al. 2007).

In contrast to cross-talk between cognate HKs and RRs, cross-regulation indicates potential hetero-pair integration of cellular processes involving multiple or distinct TCS components. Illustrative examples include TCS cross-regulation in chemotaxis (Bourret and Stock 2002) and nitrate regulation (Rabin and Stewart 1993). In *E. coli* there are 30 HKs and 32 RRs, with 5 out of the 32 not having an adjacent cognate HK-encoding gene (Mizuno 1997). Transphosphorylation between ~3% of non-cognate pairs (Yamamoto et al. 2005) implicates potential interactions between specific TCS pathways.

Evolutionary characteristics

Some bacteria such as *Mycoplasma* and *Candidatus Blochmannia floridanus* contain no TCSs, many, including *Bacillus anthracis* and *E. coli* contain several dozen and a few such as *Myxococcus xanthus* and cyanobacteria contain more than 100 (Ulrich and Zhulin 2007). Phylogenetic trees of TCS components from 14 complete and 6 partial genomes, containing 183 HKs and 220 RRs, constructed using distance methods, showed that TCSs originated in Bacteria and radiated into Archaea and Eucarya by lateral gene transfer (Koretke et al. 2000). The basic forms of two-component signaling seem to have arisen early in bacterial evolution and phylogenetic clusters. Subsequent significant diversification resulted in multiple species-specific subclusters within the major clusters. The concurrent TCSs on the chromosome supported the co-evolution model, suggesting that novel TCSs evolve by global duplication of all their components and subsequent differentiation, while eukaryotic histidine and protein kinases may show a distant evolutionary relationship (Koretke et al. 2000). In contrast, the recruitment model proposes that some TCS operons have evolved as the result of an assembly of a sensor gene and a regulator gene from heterologous TCSs. A number of studies provide supportive evidence for both models of TCS evolution (Chen et al. 2004).

Of the various signaling proteins, HKs are among the most abundant. Thus, bacteria with complex lifestyles such as *M. xanthus* and those found in varied environments such as *Pseudomonas* tend to have the largest complements of signaling proteins. In contrast, few HKs are identified in the reduced genomes of parasitic bacteria that typically live in less fluctuating environments (West and Stock 2001; Alm et al. 2006). A recent phylogenetic analysis of nearly 5,000 HKs from 207 prokaryotic genomes showed a large repertoire of recently evolved signaling genes, possibly reflecting selective pressure to adapt to new environmental conditions. Although species-specific preferences exist, lineage-specific expansion, accompanied by domain rearrangements and independent HK and RR gene evolution, as well as intact TCS horizontal gene transfer, as in *B. subtilis* and *E. coli*, contribute to the evolution of bacterial TCSs (Alm et al. 2006). This seems to allow environmental adaptation by either providing novel or retaining preexisting genetic diversity, respectively.

The structural and functional diversity of RRs is evident from the ~9,000 members that are included in databases derived from some 400 sequenced bacterial and archaeal genomes (Galperin 2006; Ulrich and Zhulin 2007). Approximately 17% of RRs have been reported to consist of a receiver domain, exhibiting 20–30% sequence identity and involved in intermolecular interactions and phosphorelays. The remainder are classified into subfamilies of mainly DNA-binding domains and to a lesser extent of enzymic, protein–protein interaction and RNA-binding domains (Gao et al. 2007). Accordingly, a recent identification and classification of 4,610 RRs, encoded in complete genomes of 200 bacterial and archaeal species, showed that almost 60% of all RRs are members of the OmpR, NarL, and NtrC subfamilies and 6% possess other DNA-binding domains (Galperin 2006). The diversity of domain architectures and the abundance of alternative domain combinations suggest that the functions of RRs are not limited to transcriptional regulation. Moreover, domain fusion may be an evolutionary mechanism allowing bacteria to regulate transcription, enzyme activity, and/or protein-protein interactions in response to environmental challenges.

Two-component systems could be associated with the evolution of bacterial species according to their environmental requirements, leading to specialized functions, including involvement in virulence, pathogenicity and host-microbe interactions. For example, a footprint of HKs and RRs from eight *B. cereus* genomes detected around 40 HK–RR gene pairs in each member and many orphan HK

and RR genes were not encoded in pairs. By comparison, *B. anthracis* appeared to lack specific HKs and RRs. *B. anthracis* evolved as a pathogen early in the evolution of the *B. cereus* group and, inhabiting relatively stable host surroundings, it may be less capable of processing extracellular signals than its close relatives exposed to more variable environments. Thus, some TCSs might have been rendered obsolete, ultimately resulting in the inactivation of HK and RR genes (de Been et al. 2006).

Two-component systems in *E. coli*

The *E. coli* genome encodes some 62 two-component proteins comprising more than 30 different TCSs that are involved in regulating diverse processes such as metabolism, transport, osmoregulation, and chemotaxis (Mizuno 1997; Oshima et al. 2002; Zhou et al. 2003. Their cross-regulation and many of their functions remain largely elusive (Mizuno 1997; Oshima et al. 2002; Zhou et al. 2003). TCSs are vital for the regulation of both constitutive and inducible processes and enable *E. coli* to orchestrate cellular economics under circumstances that might have potentially deleterious impact on their survival and well-being. Among the first TCS functions recognized was the control of catabolic genes for nitrogen and phosphorus acquisition by the NtrB-NtrC and PhoR-PhoB TCSs (Zimmer et al. 2000). Analogous imperative functions have been allocated to the AtoSC TCS.

AtoSC TCS acts directly on the transcription of the *atoDAEB* operon (Lioliou and Kyriakidis 2004; Lioliou et al. 2005), encoding for proteins involved in short-chain fatty acid (SCFA) catabolism (Jenkins and Nunn 1987a, b). Additionally, it regulates the biosynthesis of the complexed poly-(R)-3-hydroxybutyrate (cPHB) (Theodorou et al. 2006, 2007), a ubiquitous prokaryotic and eukaryotic cell constituent contributing, amongst others, to Ca^{2+} homeostasis (Reusch et al. 1995). Using phenotype microarrays, an extensive, systematic analysis of *E. coli* K-12 mutants with TCS deletions and related genes identified phenotypic differences and new roles for individual TCSs and uncovered possible interactions and cross-regulation amongst them (Oshima et al. 2002; Zhou et al. 2003).

The AtoSC two-component system

The *atoS* gene, encoding the AtoS sensor HK, and *atoC*, encoding the AtoC RR of the AtoSC TCS, are located at 49.96 and 50.00 min, respectively, in the *E. coli* genome, upstream of the *ato* operon genes *atoD*, *atoA*, *atoE* and *atoB* (*atoDAEB*) that encode proteins involved in SCFA metabolism (Fig. 1, Table 1; Blattner et al. 1997; Berlyn

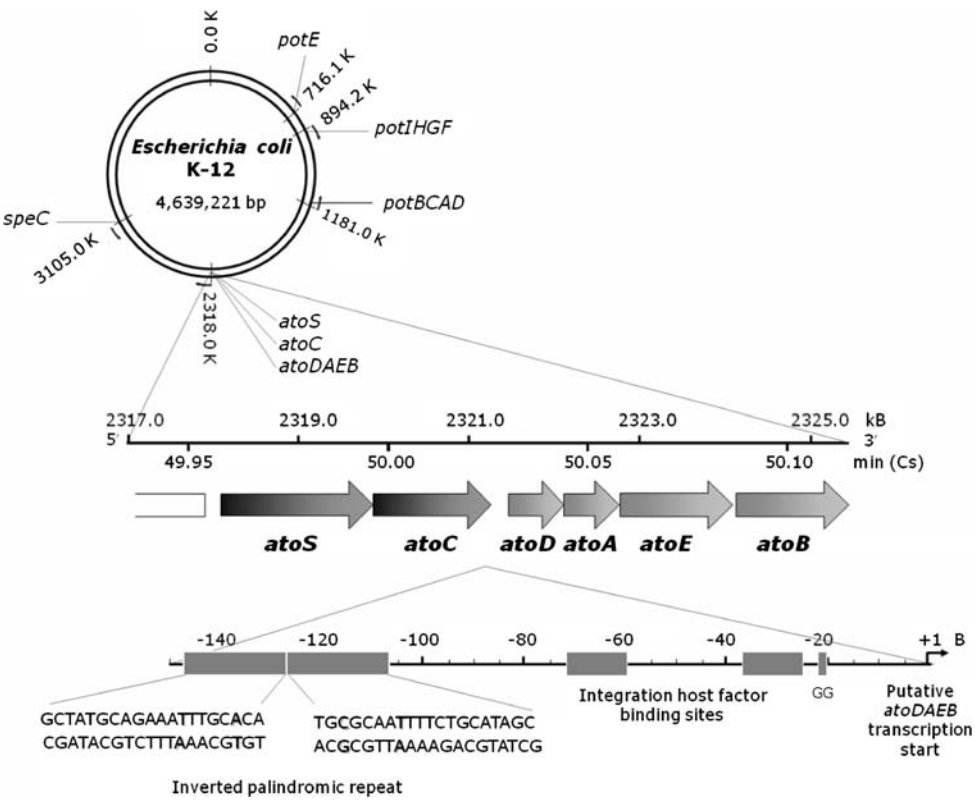

Fig. 1 Characteristics of *atoS–atoC* and *atoDAEB* operon genes in *E. coli* K-12 genome. The circular genetic map illustrates the positions of *atoS*, *atoC*, *atoDAEB*. The sizes of *atoS*, *atoC* and *atoDAEB* genes and their intergenic regions are to scale in the linear linkage map, where the *open box* and *filled arrows* indicate counterclockwise and clockwise transcription, respectively. *Gray boxes* illustrate the location, relative to the *atoDAEB* transcription start site, of DNA sequences in the transcription regulatory region of the *atoDAEB* σ^{54}-dependent promoter

Table 1 Characteristics of the *E. coli atoDAEB* operon (SWISSPROT database)

Gene	EC	Protein-activity	Comments	References
atoS	2.7.13.3	Signal transduction histidine-protein kinase (AtoS) ATP + protein L-histidine ⇆ ADP + protein N-phospho-L-histidine	Member of the AtoSC TCS Activates AtoC by phosphorylation	Canellakis et al. 1993; Itoh et al. 1996; Hayashi et al. 2006; Filippou et al. 2008
atoC		Acetoacetate metabolism regulatory protein (AtoC) Ornithine/arginine decarboxylase inhibitor Ornithine decarboxylase antizyme (Az) ODC: L-ornithine ⇆ putrescine + CO_2	Member of the AtoSC TCS Involved in transcriptional regulation of *ato* genes for acetoacetate metabolism Inhibitor of polyamine biosynthesis	Jenkins and Nunn 1987a; Canellakis et al. 1993; Theodorou et al. 2006
atoD	2.8.3.8	Acetate CoA transferase Acetyl-CoA:acetoacetate-CoA transferase α-subunit Acyl-CoA + acetate ⇆ fatty acid anion + acetyl-CoA	Heterodimer, involved in lipid, SCFA metabolism Interacts with groL Belongs to 3-oxoacid CoA-transferase subunit A family	Reed et al. 2003; Hayashi et al. 2006
atoA	2.8.3.8	Acetate CoA transferase Acetyl-CoA:acetoacetate-CoA transferase β-subunit Acyl-CoA + acetate ⇆ fatty acid anion + acetyl-CoA	Heterodimer, involved in lipid, SCFA metabolism Interacts with yfiO Belongs to 3-oxoacid CoA-transferase subunit B family	Jenkins and Nunn 1987a; Reed et al. 2003; Hayashi et al. 2006
atoE		Short-chain fatty acids transporter	Responsible for SCFA intake Ion Transporter superfamily - ST(3) structural class	Lolkema 2006
atoB	2.3.1.9	Acetyl-CoA acetyltransferase *or*Acetoacetyl-CoA thiolase 2 Acetyl-CoA ⇆ CoA + acetoacetyl-CoA	Involved in SCFA metabolism Metabolic intermediate biosynthesis; mevalonic acid biosynthesis; (R)-mevalonic acid from acetyl-CoA: step 1/3	Jenkins and Nunn 1987a

CoA coenzyme A, *EC* enzyme code, *SCFA* short-chain fatty acid, *TCS* two-component regulatory system

1998). AtoSC is a characterized TCS, which relies on AtoS HK autophosphorylation and subsequent AtoC RR phosphorylation to elicit its downstream regulatory function (Lioliou and Kyriakidis 2004; Lioliou et al. 2005; Grigoroudis et al. 2007).

Most data regarding the structural and functional characteristics of the AtoSC TCS derive from studies on various strains of *E. coli* K-12, whose complete 4,639,221 bp sequence encoding 4,288 proteins was presented in 1997 (Blattner et al. 1997). In a study using *atoSC* deletion mutants, 11 and 32 genes were up- and down-regulated, respectively, indicating the potential positive and negative roles of the system in the regulatory network (Oshima et al. 2002). Acetoacetate is the typical trigger of the AtoSC TCS that directs the expression of its downstream *atoDAEB* operon and consequently SCFA transport and catabolism (Pauli and Overath 1972; Jenkins and Nunn 1987a, b). Moreover, acetoacetate and spermidine can induce AtoSC to modulate cPHB biosynthesis (Theodorou et al. 2006, 2007). Evidence supports the contribution of AtoSC TCS chemotactic behavior (Theodorou et al. 2008a) and flagellar synthesis (Oshima et al. 2002) in *E. coli*.

Biochemical characteristics of the AtoSC two-component system

The sensor histidine kinase

AtoS transmembrane sensor HK comprises an N-terminal periplasmic ligand-binding region, a kinase domain containing the phosphorylation His398 residue in a conserved H-box, lying in proximity to the AtoC Asp55 residue upon AtoSC interaction and required for autophosphorylation and *atoDAEB* regulation, but not for ATP-binding ability, as well as a functional G2-box in the transmitter region, critical for ATP-binding (Filippou et al. 2008). The four-α-helix bundle structural motif was proposed by molecular modeling for AtoS dimerization/phosphoacceptor domain. Bioinformatic analysis indicated the occurrence of the transmembrane HAMP linker domain, present in HKs, adenyl cyclases, methyl-accepting proteins and phosphatases and the PAS and PAS-associated C-terminal domains, immediately to precede His398 at the beginning of the transmitter region (Grigoroudis et al. 2007). AtoS was shown to be in *trans*-phosphorylated between the monomers of the homodimeric protein (Filippou et al. 2008),

though *trans*-phosphorylation was hard to detect in one study possibly due to technical difficulties (Yamamoto et al. 2005). Assuming that AtoS carries a single phosphorylation site and disregarding experimental limitations, the level and rate ($t_{50\%}$, time required for half of maximal levels) of autophosphorylation at equilibrium were reported to be lower than 0.005% and more than 10 min, respectively (Yamamoto et al. 2005).

The response regulator

AtoC is a member of the NtrC-NifA family of transcriptional activators possessing intrinsic ATPase activity and requiring binding to enhancer-like element for the induction of the promoter (Grigoroudis et al. 2007; Matta et al. 2007). AtoC consists of an N-terminal receiver domain, a central AAA+ ATPase region and a DNA-binding region. The receiver domain, containing a central β-sheet of five parallel β-strands flanked by five α-helices, is linked to the σ^{54} interaction domain *via* an extended α-helical linker and communicates functionally with the ATPase region containing Walker A and Walker B motifs (Grigoroudis et al. 2007). In addition to the receiver domain Asp55 residue involved in His398 → Asp55 phosphotransfer, a potential second independent phosphorylation target has been identified at His73 residue within an unexpected H-box consensus sequence (SHETRTPV) common to homodimeric HKs (Lioliou et al. 2005; Grigoroudis et al. 2007). The possibility of His73 being part of a HK His-containing phosphotransfer domain is rather remote since AtoC bears no significant homology with these domain sequences (Dutta et al. 1999; Grebe and Stock 1999). Nevertheless, intermolecular His398 → His73 phosphotransfer and/or intramolecular Asp55/His73 interactions cannot be excluded.

Evidently, AtoC phosphorylation is required normally for AtoSC TCS to elicit plethora of its regulatory actions in vivo. However, differential molecular interactions may integrate distinct signals and downstream outputs. This may be the case in the different contribution of Asp55 and His73 residues as putative AtoC phosphoacceptor sites in AtoSC TCS-mediated regulation of the *atoDAEB* promoter (Lioliou et al. 2005) and cPHB biosynthesis (Theodorou et al. 2006). Moreover, AtoC phosphorylation by the non-cognate HK UhpB, associated with glucose-6-phosphate uptake regulation (Wright et al. 2000) implicates AtoC response to multiple signals via cross-regulation involving non-cognate HK–RR pairs (Yamamoto et al. 2005). Finally, alternative or additional modulatory mechanisms may drive these complex bacterial networks of downstream regulation. The described in vivo *atoDAEB* promoter activation in the presence of overexpressed unphosphorylated AtoC (Lioliou et al. 2005) may conform to the two-state equilibrium hypothesis, postulating that phosphorylation alters the equilibrium between active and inactive RR conformations (Volkman et al. 2001). The failure of the low molecular weight carbamoyl phosphate, phosphoramidate and acetyl phosphate to phosphorylate AtoC in vitro was suggestive of either lack of AtoC phosphorylation or its rapid dephosphorylation (Matta et al. 2007).

The atoDAEB operon

Among the AtoSC TCS positively or negatively modulated output responses (Table 2), the best characterized is the acetoacetate-activated regulation of its downstream *atoDAEB* operon. Pauli and Overath (1972) identified the closely linked *atoA* and *atoB,* encoding the β-subunit of acetyl-CoA:acetoacetyl-CoA transferase (EC 2.8.3.8), and thiolase II (EC 2.3.1.9), respectively, as the loci responsible for the SCFA acetoacetate degradation and suggested that *atoC* may encode an activator. Jenkins and Nunn (1987a) showed that the *ato* system was arranged as the *atoDAB* operon with the order of *atoD–atoA–atoB* transcribed to AtoD, AtoA, and AtoB proteins in response to the inducer. Continuing research in this field identified all genes constituting the *atoDAEB* operon (Fig. 1) and their encoded polypeptides and described a number of their structural and

Table 2 Involvement of the AtoSC two-component system in *E. coli* physiology

Activity/function	References
Activation of *atoDAEB* operon → Transcriptional regulation of short-chain fatty acid metabolism	Jenkins and Nunn 1987a; Canellakis et al. 1993; Matta et al. 2007
Poly-(*R*)-3-hydroxybutyrate biosynthesis	Theodorou et al. 2006, 2007
AtoC: (antizyme) polyamine-inducible, noncompetitive proteinaceous inhibitor of the key polyamine biosynthetic enzyme ornithine decarboxylase	Heller et al. 1976; Kyriakidis et al. 1978; Canellakis et al. 1993; Lioliou and Kyriakidis 2004
Control of flagellar gene expression in common with CitA-CitB, RcsB and YpdA-YpdB	Oshima et al. 2002
Motility and chemotactic behavior	Theodorou et al. 2008a
Increased use of glucuronamide as a carbon source. Increased sensitivity to NaCl but not KCl. Greater susceptibility to the membrane agents polymyxin B and methyltrioctylammonium chloride, the aminoglycoside dihydrostreptomycin, and the respiration inhibitor (not confirmed by dilution tests)	Zhou et al. 2003

functional characteristics particularly related to their contribution in SCFA transport and metabolism (Table 1). Both AtoSC components support *atoDAEB* induction, while reduced AtoC phosphorylation affects *atoDAEB* promoter activation, with Asp55 mutation resulting in more dramatic phenotypic alterations compared to His73 substitutions (Lioliou et al. 2005; Grigoroudis et al. 2007). Extracellular Ca^{2+} also regulates *atoDAEB* operon transcription, provided that the upstream regulatory region and the *atoD* and a part of *atoA* genes are present, implicating Ca^{2+} in AtoSC TCS signaling toward its downstream targets (Theodorou et al. 2008b).

Acetoacetate-mediated *atoDAEB* transcriptional activation in *E. coli* appears to require *cis* elements mapping within the −206 to −120 region relative to the predicted *atoDAEB* transcription start site, both in vitro and in vivo (Fig. 1). DNase I footprinting analysis revealed that AtoC binds a inverted palindromic repeat, in agreement with the relative location of other σ^{54}-dependent promoter enhancer-like elements (Reitzer and Schneider 2001). The integrity of both binding sites seems critical for AtoC to bind DNA and to exert its effects on gene expression (Matta et al. 2007). Interestingly, recent genome-wide promoter analysis combined with chromatin immunoprecipitation identified the occurrence of different putative motifs connected to this *ato* inverted palindromic repeat and indicated that AtoC may bind to more than its own promoters (Pilalis et al. 2008). The *atoDAEB* promoter contains additional characteristics of the σ^{54}-dependent bacterial promoter sequences, including two IHF binding sites at −62 to −50 and −37 to −25 and a conserved GG doublet at positions −21 and −22 (Fig. 1). Chemical mutagenesis experiments showed that the majority of mutations that impair *atoDAEB* expression are located in the conserved GG doublet. A mutation at −53, within the −62 to −50 IHF binding site and between the AtoC and RNA polymerase binding regions, reduced the inducibility of *atoDAEB* without affecting AtoC binding (Matta et al. 2007).

AtoC: the polyamine connection

In addition to its role as a transcriptional regulator, *E. coli* AtoC functions as a post-translational regulator. The *atoC* gene also encodes antizyme (Az), a polyamine-inducible endogenous non-competitive protein inhibitor of ornithine decarboxylase (ODC, L-ornithine carboxylyase, EC 4.1.1.17) that catalyzes the conversion of ornithine to putrescine in the first rate-limiting step of polyamine biosynthesis (Fong et al. 1976; Heller et al. 1976; Kyriakidis et al. 1978; Heller et al. 1983; Morris and Boeker 1983; Canellakis et al. 1993). The bifunctional role of the *atoC* gene product was hypothesized during the identification and sequencing of the gene encoding *E. coli* Az by immunological screening (Canellakis et al. 1993). The significant homologies shared between Az and sensor kinases of the TCS indicated that Az could serve as transcriptional regulator of then unknown genes, in addition to its role as inhibitor of polyamine biosynthesis. Subsequent work identified the Az as the RR of the AtoSC TCS in *E. coli*. Since its gene is identical to *atoC*, Az is now referred to as AtoC, rather alleviating strong skepticism (Ivanov et al. 1998) about its existence and function (Heller et al. 1983; Lioliou and Kyriakidis 2004).

Polyamines modulate cellular functions in both prokaryotes and eukaryotes, at least though interaction with nucleic acids and protein synthesis (Igarashi and Kashiwagi 2000; Medina et al. 2003). Besides being biosynthesized via ODC, the *potIHGF* putrescine ABC transport system located at 19.25–19.32 min, the *potBCAD* spermidine/putrescine ABC transport system at 25.45–25.51 min and a probable putrescine-ornithine antiporter encoded by *potE* at 15.44 min in the *E. coli* K-12 genome (Fig. 1; Blattner et al. 1997; Berlyn 1998) appear to operate in *E. coli*. In a complex control of their uptake and biosynthesis, ODC levels and activity are highly regulated at the transcriptional, translational, and post-translational levels. The *speC* gene, encoding ODC is located at 66.92 min in the *E. coli* K-12 genome (Fig. 1; Blattner et al. 1997; Berlyn 1998). Two ODCs have been characterized in *E. coli*. The biodegradative ODC is induced by low pH and by the presence of omithine. The biosynthetic ODC is modulated transcriptionally and post-translationally by phosphorylation and by a number of positive and negative effectors, including guanine nucleotides (Kyriakidis et al. 1978; Canellakis et al. 1981; Anagnostopoulos and Kyriakidis 1996). In an autoregulatory circuit, polyamines induce mRNA translation of Az, which then inhibits ODC activity in a stoichiometric non-competitive manner, by forming inactive complex with the enzyme (Heller et al. 1976). The mechanisms for Az regulation vary in different organisms. In mammals, the induction of Az by polyamines is due to polyamine autoregulation of a programmed ribosomal +1 frameshift, conserved in animals, fungi and protists (Ivanov and Atkins 2007).

Although AtoC has been recognized as the link between AtoSC TCS and the regulation of polyamine biosynthesis in *E. coli*, the elucidation of the mechanisms underlying their interaction(s) is limited by the scarce experimental data. Polyamines and synthetic polyamine analogs can induce *atoC* transcription resulting in increased AtoC accumulation and subsequent inhibition of ODC activity. Putrescine and the non-natural diamine diaminopropane elicited a more pronounced *atoC* transcriptional activation than spermidine and spermine, which slightly suppressed *atoC* expression, activating neither the *ato* operon promoter nor *atoS* (Filippou et al. 2007; Theodorou et al. 2007).

Likewise, the synthetic basic polyamine C48/80 showed some tendency to suppress *atoC* expression and it was unable to induce any significant alterations to *atoDAEB* (Kyriakidis et al. 2008). The variations in polyamine effects on *atoC* expression may arise from their differences in charge distribution and chain flexibility. A distance of 3-4 C-atoms between the two amino groups showed maximal *atoC* transcriptional activation and diaminopropane analogs were the most potent AtoC inducers, followed by putrescine derivatives. Spermine analogs bearing the strongly basic guanidine group on N-4 and N-9 atoms suppressed both *atoC* and *atoDAEB* operon (Filippou et al. 2007).

Physiological relevance of the AtoSC two-component system

Accumulating evidence reveals that the biological significance of the AtoSC system in *E. coli* is not limited to its role on the regulation of SCFA metabolism (Table 2; Jenkins and Nunn 1987a, b; Lioliou et al. 2005). This is an essential function of the symbiotic GI flora, which digests unutilized energy substrates, such as carbohydrates to SCFAs in favor of their host (Guarner and Malagelada 2003). Data from biochemical studies and genetic analyses continue to uncover new positive and negative direct or indirect functions for the AtoSC TCS such as regulation of cPHB biosynthesis (Rhie and Dennis 1995; Theodorou et al. 2006). Many of those are attributed to yet unresolved factors, including concentration and phosphorylation state of its cognate components in vivo, location and specificity of binding sites, and/or signaling and cross-regulation among non-cognate components and other TCSs. Illustrative examples comprise the reduced motility of Δ*atoSC* mutants, the control of flagellar gene expression, the modified expression of AtoSC components in the Δ*ompR-envZ* and Δ*yfhA* mutants and the susceptibility to specific osmolarity conditions, and membrane-interacting agents (Oshima et al. 2002; Zhou et al. 2003). Given the importance of these processes in bacterial physiology, understanding the precise AtoSC TCS functions could provide more effective approaches in many scientific areas including treatment of bacterial diseases.

Poly-(R)-3-hydroxybutyrate biosynthesis

The AtoSC TCS regulates cPHB biosynthesis in *E. coli* (Rhie and Dennis 1995; Theodorou et al. 2006). PHB, a ubiquitous constituent of prokaryotic and eukaryotic cells, is the most extensively characterized member of the carbon and energy reserve polyhydroxyalkanoates. These are of both biomedical and biotechnological interest due to their properties to modulate bacterial stress resistance and redox balance, their biodegradable character, and production by bacterial fermentation and renewable carbon sources (Madison and Huisman 1999). Studies mostly focused on *Zoogloea ramigera* and *Ralstonia eutropha* have shown PHB to be synthesized by the successive action of β-ketoacyl-CoA thiolase, acetoacetyl-CoA reductase, and PHB polymerase, encoded by the *phbCAB* operon. However, the cPHB biosynthetic pathways in *E. coli* are hitherto unresolved and most efforts have focused on PHA-producing *phb* genes expressed in recombinant *E. coli* to produce precursors of biodegradable materials (Madison and Huisman 1999).

AtoSC TCS-mediated cPHB biosynthesis can be enhanced by acetoacetate and spermidine or its N^8-acetylated form, but not by putrescine, demanding both functional AtoSC TCS and *atoDAEB* operon. As opposed to spermidine, AtoC phosphorylation at His73 is likely to be advantageous compared to that at Asp55 in the case of acetoacetate (Theodorou et al. 2006, 2007). Similarly, the synthetic basic polyamine C48/80-induced cPHB biosynthesis requires both AtoSC components. Interestingly, histamine counteracted the inductive action of C48/80 and suppressed cPHB biosynthesis, contrary to the AtoSC-controlled inductive effects of polyamines (Kyriakidis et al. 2008). Furthermore, spermidine has been shown not only to circumvent the inductive phenotype when co-administered with acetoacetate, possibly by affecting *atoDAEB* operon induction or acetoacetate transport by the AtoE membrane transporter, but also to result in cPHB redistribution from mostly cytosolic to membrane-bound, particularly during the stationary phase of growth (Theodorou et al. 2007).

Polyamine-specific functions, such as the voltage-dependent block of the K^+ channel pore (Lopatin et al. 1994) and/or altered K^+ and Ca^{2+} membrane permeability (Katsu et al. 1984; dela Vega and Delcour 1996) may contribute to the polyamine-induced phenotypes. Normally, *E. coli* do not accumulate storage polyhydroxyalkanoates, but they synthesize cytoplasmic and membrane-bound cPHB playing significant roles in a number of biological processes including Ca^{2+} homeostasis (Reusch et al. 1995; Huang and Reusch 1996). Non-proteinaceous PHB-Ca^{2+}-polyphosphate complexes are biogenerated at least via the contribution of the *acrAB*-encoded multidrug efflux pump (Jones et al. 2003). They act as voltage-gated Ca^{2+} channels with complex gating kinetics (Reusch et al. 1995; Das and Reusch 1999), high- to low-conductance state transition being important for genetic transformability or bacterial competence (Pavlov et al. 2005). Moreover, PHB-polyphosphate complex activity has been implicated in the phase of growth-dependent control of cytoplasmic free Ca^{2+} levels (Reusch et al. 1995). These findings together with the concentration- and phase of growth-dependent influence of extracellular Ca^{2+} and the inhibitory actions of the Ca^{2+}

channel blockers, with nifedipine eliciting more pronounced effects than verapamil and diltiazem on cPHB biosynthesis (Theodorou et al. 2008b) deserve consideration.

Bacterial chemotaxis

In an adaptive response to a variety of stimuli, motile bacteria achieve locomotion through swimming in liquid milieu, using mostly flagellar rotation, and gliding, swarming or twitching over solid surfaces to efficiently direct their movement, or chemotaxis, to translocate to more favorable environments (Bourret and Stock 2002).

Related research, mostly in *E. coli* and *S. enterica* established that chemotactic adaptation and signal transduction networks involve TCSs. The representative signaling circuit comprises integration of signals from multiple transmembrane chemoreceptors, termed methylated chemotaxis proteins, into a unified response through the universal coupling protein CheW that functionally links methylated chemotaxis proteins to the CheA HK. Phosphoryl group transfer from the phosphorylated CheA to the CheY or CheR RRs distributes the signal to motor components *via* the excitation pathway, involving CheA RR and the dephosphorylation accelerator CheZ or via the adaptation pathway acting through CheR RR that constitutively methylates methylated chemotaxis proteins and the phosphorylation-controlled demethylator CheB (Bourret and Stock 2002).

Evidence for the contribution of the AtoSC TCS in *E. coli* chemotactic behavior comes from data on flagellar gene expression in common with CitA-CitB, RcsB and YpdA-YpdB (Oshima et al. 2002) and from a very recent report on the requirement of both AtoS and AtoC components for *E. coli* to display the motile phenotype in solid media (Theodorou et al. 2008a). These initial data not only support the chemotactic role of the AtoSC TCS in bacterial adaptation, but they also provide an attractive starting point for the investigation of mechanisms underlying chemotactic behavior and potential cross-regulation between TCSs, such as the CheA–CheY.

Perspectives in physiology and pathophysiology

Polyamines and biogenic amines in transcriptional regulation and cell proliferation

Polyamines are ubiquitously localized in almost every mammalian cell type and elicit their biological actions mainly by direct binding to nucleic acids and intracellular proteins. They are essential not only for normal cell growth and differentiation but also for cell proliferation, neoplastic transformation, and cancer development (Canellakis et al. 1979; Igarashi and Kashiwagi 2000). On the other hand, in higher eukaryotes, physiologically import biogenic amines, such as histamine, serotonin, dopamine, and noradrenaline are involved in intercellular communication. Histamine mediates inflammation, neurotransmission, regulation of gastric acid secretion, and other vital physiological processes via binding to four types of receptors (Akdis and Simons 2006). Growing evidence implicates metabolic interplay between histamine and polyamines (Coussens and Werb 2002; Medina et al. 2003; Kyriakidis et al. 2009), among numerous other interactions. However, research on their cross-talk at the level of gene expression or metabolism is still in its infancy.

Due to their cationic nature, both histamine and polyamines are able to bind DNA, but contrary to polyamines, the potential interaction between histamine and nucleic acids has not received adequate attention (Ruiz-Chica et al. 2006). The histamine H_1 receptor antagonist chlorpheniramine, a 1,4 diamine, has been shown to bind to nucleic acids and to affect protein synthesis and ODC translation (Medina et al. 2003). Basic evidence for a potentially modulator role of histamine on gene expression came from studies on the genetically distant to mammals, albeit symbiotic microorganism *E. coli*. Histamine elicited a suppressive action on cPHB biosynthesis, in contrast to the polyamine-mediated inductive phenotype and induced *atoC* expression, without activating the *atoDAEB* operon promoter (Kyriakidis et al. 2008). It should be noted that, inverse correlation between polyamine and histamine synthesis was proposed in an expression macroarray containing human probes for biogenic amine metabolic proteins and validated on mast cells (Chaves et al. 2007).

The hitherto available data may guide ongoing research to the in-depth investigation of the potential histamine-DNA direct or indirect interactions and to the pathophysiological impact of additional, complementary, and/or distinct TCS-regulated pathways. A physiologically and pharmacologically notable hypothesis that needs to be investigated is the potential cross-regulation among AtoSC TCS, *atoDAEB* operon, their apparent modulators, particularly polyamines and biogenic amines and ionic balancing, thus offering new perspectives on bacterial growth, adaptation, and symbiotic behavior as well as on their therapeutic intervention prospect.

Bacteria–host communication

Complicated interactions are integrated in body surfaces exposed to the external environment (Guarner and Malagelada 2003; Ogra and Welliver 2008). The complex and dynamic gastrointestinal flora plays a major role in vital processes involving bacteria–host interactions. For instance, bacterial products, such as lipopolysaccharides

(LPS) may trigger selective responses during infection and inflammation (Zampeli et al. 2009), while *E. coli* may act as a factor regulating immune cell effector functions (Krämer et al. 2008).

Evidence links TCSs with quorum sensing and chemotactic gene regulation in many symbiotic bacteria including *E. coli* (Hoang et al. 2008), as well as with the adaptation of pathogens, like *Helicobacter pylori*, to their microenvironment and to drug therapies (Guarner and Malagelada 2003). Among the numerous endogenous molecules, biogenic amines appear to enable bacteria to survive and overcome host defence mechanisms, and in many occasions to elicit or arrest their pathogenic effects, often via TCS-regulated networks. Histamine was shown to modulate the defence against *E. coli* infection in experimental peritonitis (Hori et al. 2002). The enterohemorrhagic *E. coli*, which is responsible for worldwide outbreaks of gastrointestinal manifestations, activates transcription of genes involved in intestinal lesions, such as the QseEF TCS, in response to signals originating from intestinal microflora and the host's epinephrine and norepinephrine (Reading et al. 2007). Along this line of evidence and taking into consideration that, distorted symbiotic communication may lead to pathological manifestations, the cross-regulation between biologically important amines and TCS networks that may lead to favorable bacteria–host symbiosis, predominantly in the gastrointestinal tract where histamine has decisive physiological roles, is a tempting hypothesis.

Resistance to drugs

A number of studies using mainly pathogenic microorganisms demonstrated the involvement of TCSs in drug resistance. Amongst these, CzcR-CzcS participates in imipenem resistance in the opportunistic pathogen *Ps. aeruginosa* (Perron et al. 2004). Changes in sensitivity to penicillin, vancomycin, and ethambutol are mediated by MtrA-MtrB in *Corynebacterium glutamicum* (Möker et al. 2004) and VanR-VanS activates the expression of *van* genes that confer resistance to vancomycin in *Streptomyces coelicolor* (Hong et al. 2005). Sequence similarity studies have shown the existence of at least 32 RR genes in *E. coli*. Fifteen of them, *baeR*, *citB*, *cpxR*, *evgA*, *fimZ*, *kdpE*, *narL*, *narP*, *ompR*, *rcsB*, *rstA*, *torR*, *yedW*, *yehT*, and *dcuR*, conferred increased single- or multi-drug resistance, two-thirds conferring deoxycholate resistance, while five genes, *evgA*, *baeR*, *ompR, cpxR,* and *rcsB*, modulated the expression of several drug exporter genes (Hirakawa et al. 2003).

TCS differential activity and cross-regulation appear to underlie the divergent virulence and resistant phenotypes to antibiotics in distinct pathogens. This is illustrated by the differential activation, even in phylogenetically close species, of the PmrA-PmrB TCS that directly controls *pmrF* polymyxin-resistance operon and regulates LPS modifications that enable Gram-negative bacteria to exhibit resistance to the cationic peptide antibiotic polymyxin B (Fu et al. 2007). In *S. enterica*, polymyxin B resistance is governed by *pmrF* expression mediated by both PmrA-PmrB and PhoP-PhoQ that are functionally linked through the polymyxin resistance protein PmrD, whereas *E. coli* K-12 appears to have adopted a different strategy where PmrD connectivity between the two TCSs is blocked (Fu et al. 2007). Regulation diversity is further evidenced by the PmrA-PmrB-independent *pmrF* expression and the PhoP direct action on *pmrF* in the enteropathogen *Yersinia pseudotuberculosis* (Marceau et al. 2004) as well as by the polyamine-induced *phoPQ* expression, linked to quinolone, and polymyxin B resistance in *Ps. aeruginosa* (Kwon and Lu 2006). However, spermine showed synergistic effects in β-lactam susceptibility of *E. coli*, *S. enterica* and in the Gram-positive *S. aureus* including the methicillin-resistant MRSA strain (Kwon and Lu 2007). The expression of the polyamine transporter *pot* operon, implicated in pneumococcal pathogenesis, was suppressed in response to penicillin in *Streptococcus pneumonia* (Rogers et al. 2007). These indicative findings suggest that, in addition to the complexity of TCS cross-regulation in mediating drug-resistant phenotypes in bacteria, complicated molecular mechanisms may be implicated in polyamine-mediated resistance or susceptibility to antibiotics.

In *E. coli*, polyamines block the entrance of certain β-lactams through the OmpF and OmpC outer membrane porins (dela Vega and Delcour 1996), while efflux pumps such as AcrAB can extrude antibiotics, increased efflux pump activity being one of the major mechanisms for multidrug resistance (Nikaido 1996). Interestingly, the AcrAB multidrug efflux pump contributes to PHB–Ca^{2+}–polyphosphate complex biosynthesis (Jones et al. 2003) and spermidine enhances AtoSC TCS-mediated cPHB biosynthesis and promotes cPHB redistribution (Theodorou et al. 2007). Therefore, although *ato* genes have not been identified amongst those conferring single- or multi-drug resistance in a sequence similarities study of 32 RR genes (Hirakawa et al. 2003), it would be interesting to investigate the potential role of the AtoSC TCS in the drug resistance or sensitivity phenotypes and the possible role of polyamines in this regulatory network.

Concluding remarks

Extracellular information is converted into an exploitable intracellular form via signal transduction. This critically

important process occurs in both unicellular and multicellular organisms throughout the living world and is elicited in prokaryotic and eukaryotic cells comparably. The mechanistic details of information processing vary from case to case. Diverse systems, nevertheless, display a number of operating principles in common. Thus, the study of one signaling system can yield insights applicable to others. This overview portrays one of the well-characterized examples of information processing in bacteria, the AtoSC two component signal transduction system. By governing the *ato* operon, the AtoSC TCS may lead to different homeostatic cellular biosynthetic mechanisms, such as cPHB and polyamines, or to rather more complicated adaptive responses, such as chemotaxis, balancing of Ca^{2+}, K^{+}, glucose or polyamine fluxes, and possibly to many more. Using AtoSC as an example, primarily we endeavored to summarize what has been learned so far of general interest and, additionally, we pointed out some features that still are not entirely understood.

A model for the role of the AtoSC system and the cascade of reactions that are triggered by its activation are shown in Fig. 2. Acetoacetate, acetoacetyl-CoA, spermidine, or other amine analogs act as signals upon which the AtoS HK is autophosphorylated. Subsequently, the phosphoryl group is transferred by protein-protein interaction to the AtoC RR. AtoC is thus activated and able to bind to the *atoDAEB* operon promoter at an inverted palindromic repeat (Fig. 1). Thereafter, AtoC is oligomerized, a process that triggers its ATPase activity and in that way the specific signal initiates the formation of high-order oligomers that regulate a number of cellular processes. Upon ATP hydrolysis, the closed form of the RNA-σ^{54} holoenzyme is transformed into an open form, capable to induce *atoDAEB* operon transcription, which leads to SCFA catabolism and activation of the cPHB biosynthetic pathway. A functional AtoSC system appears to affect *E. coli* chemotactic behavior, while some other putative roles related to adaptive responses are under investigation.

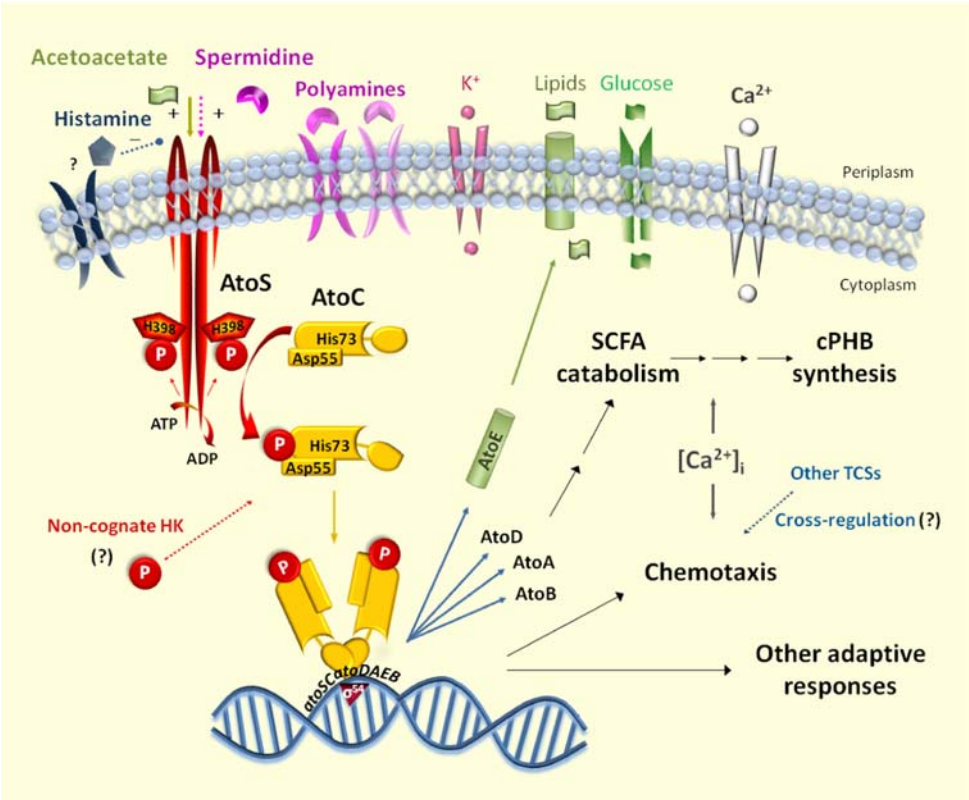

Fig. 2 Functions of AtoSC TCS. Acetoacetate or spermidine generates the signal for homodimeric AtoS sensor HK autophosphorylation at His398, which is followed by phosphoryl group transfer to Asp55 and/or possibly to the His73 residue of the AtoC response regulator. Activated dimerized AtoC binds to the promoter of the *atoDAEB* operon, encoding acetate CoA transferase α- (AtoD) and β- (AtoA) subunits, acetoacetyl-CoA thiolase (AtoB) and short-chain fatty acid transporter (AtoE) and induces transcription *via* the action of σ^{54} holoenzyme RNA polymerase, leading to SCFA catabolism, activation of the cPHB biosynthetic pathway, modulation of chemotactic behavior and other yet undefined adaptive responses. Ca^{2+} modulates these responses and histamine elicits a putative suppressive action, while the role of polyamine transport, K^{+} balancing and the cross-regulation involving other TCSs deserve consideration

In an attempt to understand its pleiotropic roles in symbiosis, we propose possible functions of the unique constituents of the AtoSC system, the AtoS and AtoC proteins, promotors, or other elements. The possible role of AtoC phosphorylation in ODC regulation is also under investigation. To date, it is not clear whether mammalian antizymes possesses properties similar to AtoC and comparable transcriptional regulator function. Furthermore, the actual role of polyamines, histamine, and various metabolites in activating the AtoSC TCS remains to be elucidated. This may bring into interplay two apparently unrelated biological pathways, for instance SCFA catabolism and polyamine biosynthesis.

Uncertainties concerning TCS interplay and the actual role of the AtoS and AtoC must be further clarified before it can be considered worthwhile to extend the scope of research in this particular area. The investigation of the initial autophosphorylation of the AtoS HK may encourage the development of specific inhibitors that block this simple reaction, and consequently the initial signal, leading to the identification of selective and efficient novel pharmacological targets. Finally, the emerging concept of more complex structures such as regulons that are constantly remodeled in vivo, favoring adaptation, survival, growth, and homeostatic mechanisms is an attractive perspective in TCS research.

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