Let LuxS speak up in AI-2 signaling

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Quorum sensing is a process of bacterial cell–cell communication that uses small diffusible molecules to coordinate diverse behaviors in response to population density. The only quorum-sensing system shared by Gram-positive and Gram-negative bacteria involves the production of autoinducer-2 (AI-2). The AI-2 synthase LuxS is widely distributed among the Bacteria, which suggests that AI-2 is a language for interspecies communication. However, LuxS is also an integral component of the activated methyl cycle in bacteria. LuxS-based quorum sensing has been intensively studied in the past decade, mostly in relation to the AI-2 molecule and the downstream effects of *luxS* knock-outs; few studies have focused on the gene and protein activity itself. Ongoing attempts to dissect the metabolic and signaling roles of LuxS leave little doubt that unraveling the regulation of *luxS* expression and cellular LuxS activity is the key to understanding LuxS-based quorum sensing.

The playground of the LuxS enzyme: intriguing yet not delineated

Bacteria regulate gene expression in response to a variety of extracellular signals to enable them to adapt to different environmental conditions. Signals can be generated by the bacteria themselves as a function of their population density and many bacteria use these molecules to communicate and coordinate social activities, a process referred to as quorum sensing [1]. Initially, it was believed that the signaling molecules produced by one species served for private conversations (i.e. communication within that same species). However, in the early 1990s, Bassler *et al.* [2] discovered that the free-living marine bacterium *Vibrio harveyi* uses two different signaling molecules to regulate bioluminescence, one of which is autoinducer 2 (AI-2), a novel signaling molecule that seemed to be designed for interspecies communication. Indeed, database searches revealed the presence of a *luxS* homolog – the biosynthetic gene for AI-2 – in many other Gram-negative and Gram-positive bacterial species [3,4]. *luxS* mutants have been constructed in different species, and phenotypes such as bioluminescence (discovered first, hence the name *luxS*), regulation of virulence factors and biofilm formation have been described [4–6].

Although LuxS was identified in 1999 [3], Winzer *et al.* [7] drew attention to the fact that LuxS had already been described in the 1960s [8] as the *S*-ribosylhomocysteine (SRH) cleavage enzyme [9]. This enzyme has an important

metabolic function in the activated methyl cycle, namely the recycling of *S*-adenosylhomocysteine (SAH) to homocysteine, although an alternative pathway does exist (Figure 1). This could also explain why LuxS is conserved among ~50% of the sequenced bacteria [7,10,11].

In a recent review, Vendeville *et al.* [5] clearly documented the two potential roles of LuxS and AI-2 in metabolism and quorum sensing. In our opinion, the current knowledge about the regulation of *luxS* and LuxS activity is insufficient to assign a signaling role to AI-2 in all of the bacteria in which AI-2 has been detected so far. Furthermore, we suggest approaches for future experiments that take into account the possible roles of small non-coding RNAs in the regulation of *luxS*-related phenotypes and the role of post-translational processing in regulating LuxS activity.

The role of LuxS as AI-2 synthase

AI-2 is produced from *S*-adenosylmethionine (SAM) in at least three enzymatic steps [12] (Figure 1a). Consumption of SAM as a methyl donor produces *S*-adenosylhomocysteine (SAH), which is subsequently detoxified by the nucleosidase Pfs to yield adenine and *S*-ribosylhomocysteine (SRH). SRH is converted to 4,5-dihydroxy-2,3-pentanedione (DPD) and homocysteine. This reaction is catalyzed by LuxS [9]. DPD spontaneously rearranges into AI-2 [12]. It was reported that, in *Escherichia coli*, LuxS is also involved in the synthesis of another, as yet uncharacterized, signaling molecule AI-3, which cross-communicates with the epinephrine host signaling system [13].

Although >55 bacterial species have a *luxS* homolog, only two AI-2 structures have been elucidated: a furanosyl borate diester (3A-methyl-5,6-dihydro-furo(2,3-*D*)(1,3,2)dioxaborole-2,2,6,6A-tetraol; *S*-THMF-borate) in *V. harveyi* [14] and (2*R*,4*S*)-2-methyl-2,3,3,4-tetrahydroxy-tetrahydrofuran (*R*-THMF), which lacks boron, in *Salmonella typhimurium* [15]. The molecules DPD, *R*-THMF and *S*-THMF-borate are in equilibrium with one another (Figure 1b). ‘AI-2’ should, therefore, be used as a collective term for DPD-derived and interconvertible molecules that can promote cross-communication [16]. What about AI-2 molecules in other bacteria? It is assumed that these are *R*-THMF or *S*-THMF-borate, or at least, similar molecules. The more knowledge that we have on the different members of the AI-2 molecule family, the more insight we will gain on the proposed role of this molecule. To this end, more input is required from structural biologists and chemists. Until now, only a few cases with direct cell–cell signaling processes related to LuxS have actually been demonstrated (for recent review, see Ref. [5]), for example,

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Available online 3 February 2006

TRENDS in Microbiology

Figure 1. DPD biosynthesis in relation to the activated methyl cycle. **(a)** Following methyl transfer from SAM to its various substrates, SAH is formed. SAH is toxic and is removed in two ways, either using a two-step enzymatic conversion by the Pfs and LuxS enzymes, producing adenine, homocysteine and DPD, or a one-step conversion using SAH-hydrolase (SahH). The latter pathway exists in some organisms and it is rare to have components of both. Homocysteine is used to recycle methionine. In *Escherichia coli*, two enzymes catalyze the transfer of the methyl group of N⁵-methyltetrahydropteroyl glutamate (CH₃-THPG) to the sulfur of homocysteine to form methionine. One of these, the cobalamin-dependent methionine synthase (Meth) can also use N⁵-methyltetrahydrofolate (CH₃-THF) as a methyl donor, whereas the cobalamin-independent methionine synthase (MetE) is restricted to CH₃-THPG. Methionine is converted to SAM in a reaction catalyzed by SAM synthetase (MetK). **(b)** DPD undergoes a thermodynamically favorable cyclization to either the *R* or *S* form of 2,4-dihydroxy-2-methyldihydro-3-furanone (DHMF). *R*-DHMF can then undergo hydration to form *R*-THMF, the AI-2 molecule of *Salmonella typhimurium* [15]. Hydration of *S*-DHMF can form *S*-THMF, which can subsequently form a diester with boric acid to generate *S*-THMF-borate, the AI-2 molecule of *Vibrio harveyi* [14]. Double arrows indicate the reversible nature of interconversions. Based on data from Refs [5,7,8,12,15,19].

in *Vibrio* species [17]. Further work must be carried out to determine if LuxS does actually influence intercellular signaling. The availability of pure DPD through chemical synthesis has already been useful in this respect [18,19].

LuxS and recycling of S-adenosylhomocysteine (SAH)

LuxS has an important function in the activated methyl cycle of the cell because it is necessary for recycling of the toxic intermediate SAH [7] (Figure 1). Because of its structural similarity to SAM, SAH is a potent feedback inhibitor of the SAM-dependent methyltransferases [20]. Except for some symbionts and parasites, all organisms have a pathway to recycle SAH, either using a two-step enzymatic conversion by the Pfs and LuxS enzymes to produce adenine, homocysteine and DPD, or a one-step conversion using SAH hydrolase (SahH) (Figure 1). There is no AI-2 production through the SAH-hydrolase pathway, which is most common in Eukaryotes, Archaea, α -proteobacteria, Actinobacteria and Cyanobacteria [10,11]. Interestingly, *Bifidobacterium longum* NCCC2705 and *Escherichia blattae* have both pathways [11] and, hence, are interesting strains in which to investigate the signaling role of LuxS. However, to our

knowledge, AI-2 has not yet been demonstrated in these species.

luxS mutants

The function of AI-2 in different species has been studied by constructing *luxS* mutants and scoring for phenotypes that could be complemented by adding conditioned medium of the corresponding wild-type strain. However, conditioned media are not the proper AI-2 source for complementation assays. Conditioned media prepared from wild-type and *luxS* mutant strains are likely to differ not only in AI-2 but also in many other aspects (e.g. because of the disruption of the activated methyl cycle) [7,21]. Therefore, studies on the effect of *luxS* should include both genetic and chemical complementation (although this is often neglected; see Ref. [5]) using chemically defined molecules [18,19]. However, chemical complementation might need to take into account the timing and production (threshold) level of AI-2 *in vivo* but quantitative data on these parameters are not yet available.

Each organism that contains *luxS* has only a single copy of the gene [10,11]. Nevertheless, although LuxS is

Figure 2. Sequence alignment of SraD homologs. A sRNA is located upstream of *luxS* of *Escherichia coli*, designated *sraD* [26]. This sRNA is conserved in other bacterial species [27]. In all of these species, *sraD* is located upstream of the *luxS* homolog on the complementary strand. BLASTN was used to screen for *sraD* homologs in completely sequenced genomes. Numbers to the left of the sequence refer to: 1, *E. coli* K-12 MG1655; 2, *Shigella sonnei* Ss046; 3, *E. coli* CFT073; 4, *E. coli* O157:H7; 5, *Shigella flexneri* 2a; 6, *Salmonella enterica* subsp. *enterica* serovar Paratyphi A; 7, *Salmonella enterica* subsp. *enterica* serovar Choleraesuis; 8, *Salmonella enterica* serovar Typhi strain CT18; 9, *Salmonella typhimurium* LT2; 10, *Serratia marcescens*; 11, *Serratia plymuthica*; 12, *Yersinia pestis* biovar Medievalis; 13, *Yersinia pseudotuberculosis* IP32953; 14, *Yersinia pestis* strain CO92; 15, *Yersinia pestis* strain KIM; 16, *Erwinia carotovora* subsp. *atroseptica* SCRI1043; 17, *Erwinia carotovora* subsp. *carotovora*. Black shading indicates that nucleotides are conserved in all aligned sequences, dark gray indicates conserved nucleotides in at least 80% of the aligned sequences and light gray indicates conserved nucleotides in at least 60% of the aligned sequences.

involved in the activated methyl cycle, *luxS* mutants do not show a growth defect in most cases [22,23]. Interestingly, *luxS* mutants of some species have a specific effect on gene expression (e.g. virulence factor regulation in *V. cholerae* [17]), whereas others show no change or a subtle change in phenotype (e.g. *E. coli* K-12 [21]). Also, the type of *luxS* mutation in a given species can result in a different phenotype. A *Streptococcus mutans luxS* deletion mutant shows altered biofilm formation as compared with the wild type [24], whereas a *S. mutans luxS* insertion mutant does not [25]. This apparent inconsistency could be a result of different experimental methods, as suggested by the authors [24]. Alternatively, important regulatory elements that overlap with the coding sequence of *luxS* could be present and might be disturbed in a deletion mutant but not in an insertion mutant. Another example can be found in *S. typhimurium*, in which a *luxS* deletion mutant impaired in biofilm formation can be rescued by the introduction of *luxS* under control of its own promoter but not under control of a constitutive promoter [19]. Are regulatory elements in the promoter region of *luxS* involved? In *E. coli*, a small regulatory RNA (sRNA), *sraD*, is located upstream of *luxS* with its promoter region overlapping with the *luxS* coding sequence [26]. This sRNA is conserved in closely related species [27] such as *S. typhimurium* (Figure 2). In bacteria distantly related to *E. coli*, *sraD* is absent, which would imply a different form of regulation such as the riboswitches observed in some Gram-positives [28]. Alternatively, it might be that the *sraD* homologs in other species are less conserved and, thus, have not yet been identified. Although sRNAs have been reported to control quorum sensing in some species [29,30], the role of the sRNA upstream of *luxS* in the regulation of LuxS-related phenotypes has not yet been studied. sRNAs can regulate both the synthesis of proteins (by affecting mRNA transcription, translation and stability) and the activity of specific proteins (by binding to them) [31,32]. These sRNAs regulate bacterial genes involved in environmental adaptation [32], which makes them of interest as putative members of the LuxS-AI-2 quorum-sensing system. Indeed, *luxS*-controlled gene expression varies with environmental conditions [21].

Motifs in LuxS

LuxS is a small metalloenzyme (± 170 amino acids). Sequence alignment (Figure 3) reveals an invariant His-Xaa-Xaa-Glu-His motif, which is often found in Zn^{2+} -containing proteins. The crystal structures of LuxS from several bacterial species have been determined to high resolution [9,33,34]. These studies revealed that LuxS exists as a homodimer and that two identical active sites are formed at the dimer interface by residues from both subunits. Each active site contains a divalent metal ion, which was initially reported to be Zn^{2+} [9,33] but was subsequently shown to be Fe^{2+} [35]. This Fe^{2+} catalyzes an internal redox reaction that shifts the ribose carbonyl group from its C1 to C3 position. Subsequent β -elimination completes this highly unusual reaction [35,36].

Further inspection of the LuxS sequence of *V. harveyi* (Q9Z5X1), the species in which AI-2 signaling has been demonstrated unambiguously [5], reveals yet another motif (Figure 3). *In silico* analysis revealed the presence of the sequence Lys-Ile-Pro-Glu-Leu-Asn-Glu-Tyr, which matches the consensus sequence of a tyrosine kinase phosphorylation site: [Lys/Arg]-Xaa₂₋₃-[Asp/Glu]-Xaa₂₋₃-Tyr (according to Prosite PS00007 [37]). Phosphorylation is a covalent modification that proteins undergo frequently in post-translational processes and is of major importance in the cellular activities of microorganisms. Protein phosphorylation on tyrosine is a phosphorylation type that was long considered to be specific to eukaryotes. In recent years, however, conclusive evidence has accumulated that shows it also occurs in prokaryotes [38]. Extended *in silico* screening for tyrosine-phosphorylation-site motifs in known LuxS proteins showed that several of these proteins in Gram-negatives (e.g. *S. typhimurium*) do have a conserved tyrosine phosphorylation site motif that gives a nearly perfect match to the consensus sequence (Figure 3).

Tyrosine phosphorylations are associated with the production of exopolysaccharides in several bacterial pathogens [39]. In several organisms including *Salmonella* species [19,40], the production of exopolysaccharides and *luxS* expression have been linked with biofilm formation. Nevertheless, post-translational modifications

Figure 3. Sequence alignment of LuxS homologs of different species. LuxS is conserved in many bacterial species and is a small metalloenzyme (± 170 amino acids). Sequence alignment reveals an invariant His-Xaa-Xaa-Glu-His motif (green box), which is important in the catalytic activity of LuxS [35]. *In silico* domain screening suggested the presence of a semi-conserved tyrosine kinase phosphorylation site: [Lys/Arg]-Xaa₂₋₃-[Asp/Glu]-Xaa₂₋₃-Tyr (according to Prosite PS00007 [37]; P site, indicated by yellow box). Numbers to the left of the sequence refer to: 1, *Escherichia coli* K-12 MG1655; 2, *Shigella sonnei* Ss046; 3, *E. coli* CFT073; 4, *E. coli* O157:H7; 5, *Shigella flexneri* 2a; 6, *Salmonella enterica* subsp. *enterica* serovar Paratyphi A; 7, *Salmonella enterica* subsp. *enterica* serovar Choleraesuis; 8, *Salmonella enterica* serovar Typhi strain CT18; 9, *Salmonella typhimurium* LT2; 10, *Serratia marcescens*; 11, *Serratia plymuthica*; 12, *Yersinia pestis* biovar Medievalis; 13, *Yersinia pseudotuberculosis* IP32953; 14, *Yersinia pestis* strain CO92; 15, *Yersinia pestis* strain KIM; 16, *Erwinia carotovora* subsp. *atroseptica* SCRI1043; 17, *Erwinia carotovora* subsp. *carotovora*. The alignment was performed using ClustalW 1.8 [49] and edited in GeneDoc [50]. Black shading indicates that residues

of LuxS (such as phosphorylation) and their effects on activity have not been studied in this enzyme.

Regulation of *luxS* expression

Some studies have been published on the regulation of *luxS* expression in *E. coli* and *S. typhimurium*. In *E. coli*, *luxS* transcription is induced by acid [41,42]. Moreover, *E. coli* AI-2 synthesis is subject to catabolite repression through the cAMP–cAMP receptor protein (cAMP–CRP) complex, which indirectly represses *luxS* expression: the cAMP–CRP complex does not bind to the *luxS* promoter [23]. The expression of *pfs* was decreased in the presence of glucose; however, this probably occurs through a cAMP-independent mechanism [23]. The effect of glucose on *luxS* transcription is reflected in the LuxS protein level [43].

By contrast, transcription and translation of *S. typhimurium luxS* is reported to be constant through all phases of growth [44], and the profile of *pfs* transcription is tightly correlated to the AI-2 production pattern, although it seems not to be regulated by AI-2. These results suggest that AI-2 production is regulated at the level of LuxS substrate (SRH) availability. Therefore, the conclusion is that AI-2-dependent signaling in *S. typhimurium* is a reflection of the metabolic state of the cell and not of cell density [44]. Also, in *Streptococcus bovis*, *luxS* transcription is not directly related to cell density [45]. However, in contrast to the *S. typhimurium luxS*, *S. bovis luxS* expression is not constitutive but is linked to the availability of glucose in the medium and the growth rate of the cells [45].

Only a few regulatory elements of *luxS* have been described across all of the species examined and no direct binding of regulatory proteins to the promoter region of *luxS* has been shown. However, because the phenotypes of *luxS* mutants reported so far are observed under specific conditions (e.g. biofilm formation by *S. typhimurium* [19,40]), the regulation of *luxS* transcription needs to be studied under those specific conditions.

Relationship between LuxS and AI-2 levels

Usually, *luxS* expression is studied indirectly by determining the extracellular AI-2 levels in different environmental and genetic conditions. However, as mentioned earlier, the transcription profile of *luxS* during bacterial growth does not coincide with the accumulation profile of AI-2 in the bacterial culture fluid. In most bacteria that were examined, extracellular AI-2 activity peaks in mid-to-late exponential phase and declines precipitously in stationary phase. In *S. typhimurium* [22,46] and *E. coli* [47], the rapid disappearance of AI-2 is a consequence of its import by an ATP-binding cassette (ABC) transporter designated the *luxS*-regulated (Lsr) transporter (Box 1). Antibodies to the LuxS protein showed that LuxS was present even during late stationary phase despite drastically reduced levels of extracellular AI-2 [43,44,47]. Moreover, the absence of increased levels of AI-2 upon overproduction of LuxS is a result of a lack of

are conserved in all aligned sequences, dark gray indicates conserved residues in at least 80% of the aligned sequences and light gray indicates conserved residues in at least 60% of the aligned sequences.

Box 1. AI-2 regulated genes in *Salmonella typhimurium*

In *Salmonella typhimurium*, AI-2 is produced and released during exponential growth and is subsequently imported into the bacteria by the Lsr ATP-binding cassette (ABC) transporter [46]. AI-2 induces transcription of the *lsrACDBFGE* operon, the first four genes of which encode the Lsr transport apparatus. LsrK is required for the regulation of the *lsr* operon and the AI-2 uptake process. LsrK is a kinase that phosphorylates AI-2 upon entry into the cell [22]. Phosphorylation of AI-2 results in its sequestration in the cytoplasm. Taga *et al.* [22] suggest that phospho-AI-2 is the inducer responsible for inactivation of LsrR, the repressor of the *lsr* operon. LsrF and LsrG are necessary for the further processing of phospho-AI-2. Transport and processing of AI-2 could be required for termination of AI-2 signaling or for interfering with the AI-2 signaling of other species [16]. In *S. typhimurium*, no AI-2-regulated genes other than those in the *lsr* operon have yet been identified. Nevertheless, an *S. typhimurium luxS* mutant has been reported to be defective in biofilm formation *in vitro* [19] and on gallstones [40]. Interestingly, neither AI-2 nor the activated methyl cycle substrates or intermediates seem to be major players in *S. typhimurium* biofilm formation [19] because biofilm formation of an *S. typhimurium luxS* mutant could not be restored by addition of DPD, methionine or SAM [19]. Moreover, genetic complementation of biofilm formation by the *S. typhimurium luxS* mutant could only occur when *luxS* was introduced under the control of its own promoter [19]. This suggests that the promoter region of *luxS* contains important regulatory elements for *S. typhimurium* biofilm formation.

SRH availability [43,44].

The sustained presence of AI-2 in stationary-phase culture supernatants requires the presence of glucose in the growth medium [43,48]. In the presence of glucose (or other phosphotransferase system sugars), the *lsr* operon is not transcribed because of catabolite repression, thereby preventing rapid AI-2 uptake. Also, other mechanisms are involved in the regulation of Lsr-mediated AI-2 uptake (Box 2). Interestingly, besides the *lsr*-encoded AI-2 uptake system, a low-affinity AI-2 transporter in *E. coli* and *S. typhimurium* also exists that has not yet been characterized [22,23,47] (Box 2).

AI-2 uptake systems have not been studied in detail in other bacteria. Depending on the role of LuxS in a communication system or in the activated methyl cycle, different explanations are postulated as to why bacteria

produce and secrete AI-2 only to take it up again later. One possibility is that uptake of AI-2 could serve to disrupt communication between other bacteria [16]. This could also be the function of the Lsr-transporter homolog found in *Sinorhizobium meliloti*, which has no *luxS* gene [11]. From a different point of view, AI-2 could be taken up again in the later stages of growth to avoid the loss of metabolically 'expensive' compounds [20].

Other players in the LuxS playground

An exciting development in recent years is the discovery that bacteria 'talk to each other', thereby mimicking a multicellular organization. The AI-2 signal molecule is a fascinating signal because it is produced and interpreted by a variety of bacteria [4]. Substantial research has already provided insight into the complex AI-2-mediated processes. However, there is an assumption that, if a *luxS* mutant has a phenotype, then cell-cell signaling must be involved in regulating that phenotype. Suitable experimental criteria to make more definite conclusions have been suggested previously [5,20], such as the use of chemically synthesized DPD and the construction of strains that do not have a mechanism to sense or respond to exogenous AI-2. Moreover, future experiments should include the characterization of the identity of the AI-2 molecule and determination of the receptor and detection cascade involved in a specific species. We feel that, in parallel with continuing the search for the role of AI-2 in signaling, the AI-2 synthase itself and its regulation should be analyzed in depth, with cells grown under different conditions (e.g. planktonic versus biofilms). The involvement of sRNA and post-translational modifications must be investigated in view of the intriguing signatures for these regulatory mechanisms upstream of the gene that encodes *luxS* and on the LuxS protein. These approaches are also indispensable for estimating the potential of LuxS in the development of effective antimicrobial strategies through interference with AI-2 mediated processes.

Box 2. Regulation of AI-2 uptake in *Escherichia coli* and *Salmonella typhimurium*

Both cAMP–CRP-dependent and -independent mechanisms regulate AI-2 uptake in *Escherichia coli* and *Salmonella typhimurium*. Specifically, cAMP–CRP is shown to bind to a CRP binding site located in the upstream region of the *E. coli lsr* promoter [23]. Thus, AI-2 cannot be imported and it accumulates in cell-free culture fluids. In the absence of glucose, AI-2 is produced but its presence is transient as a result of rapid internalization by the Lsr transporter [47]. Also in *S. typhimurium*, mutation of *cya* or *pstI* – two genes that indirectly affect catabolite repression – caused reduced transcription of the *lsr* operon [22]. In an *E. coli glpD* mutant, AI-2 uptake is repressed. *glpD* encodes the glycerol-3-phosphate (G3P) dehydrogenase, which is involved in glycerol and G3P metabolism. G3P accumulates in the *glpD* mutant and represses *lsr* transcription by preventing cAMP–CRP-dependent activation [47].

Besides cAMP–CRP, the repressor LsrR controls expression of the *lsr* operon and, thus, AI-2 uptake in *E. coli* and *S. typhimurium*. Moreover, in *E. coli*, *lsrR* transcription is autorepressed [21]. Upon entry into *S. typhimurium* (and probably into *E. coli* [47]), AI-2 is phosphorylated by the AI-2 kinase LsrK [22] (Box 1). The phospho-AI-2 seems to be the inducer that releases LsrR-mediated repression of *lsr* transcription

[22]. Until now, LsrR was not shown to bind to the promoter of the *lsr* operon and it is not clear whether the effect of phospho-AI-2 on LsrR is direct. Recently, it was reported that dihydroxyacetone phosphate (DHAP) or a derivative represses *E. coli lsr* transcription by a cAMP–CRP-independent mechanism [47]. DHAP is formed by the oxidation of G3P and is further metabolized through the glycolytic pathway. It was suggested that DHAP could function as an anti-inducer of the *lsr* operon by inhibiting the binding of phospho-AI-2 to LsrR, which could cause LsrR to remain locked in its active, repressing state [47]. In *S. typhimurium*, LsrF and LsrG are involved in modifying phospho-AI-2 [22] (Box 1). It is possible that one of the modification products is DHAP because pentose phosphates are often converted to DHAP to be channeled to the glycolytic pathway for further metabolism [47].

Other regulators of AI-2 uptake are RpoS in *E. coli* [23] and the Lon protease in *S. typhimurium* [22] but the relation of these regulators to the regulation of *lsr* expression is less clear.

Besides the *lsr*-encoded AI-2 uptake system, a low-affinity AI-2 transporter also exists in *E. coli* and *S. typhimurium* [22,23,47], which is subject also to G3P catabolite repression [47].

Acknowledgements

We apologize to the many researchers studying AI-2 and LuxS whose publications could not be cited owing to space limitations. This work was supported by the IWT-Vlaanderen through research project GBOU-20160.

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