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ColR-ColS signalling system and transposition of Tn*4652* in the adaptation of *Pseudomonas putida*

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I **Kivistik PA, Kivisaar M, Hõrak R**: Target site selection of *Pseudomonas putida* transposon Tn*4652*. J Bacteriol 2007, 189:3918–3921.

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III **Kivistik PA, Kivi R, Kivisaar M, Hõrak R**: Identification of ColR binding consensus and prediction of regulon of ColRS two-component system. BMC Mol Biol 2009, 10:46.

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My contribution to journal articles of the current dissertation is following:

Ref. I – I planned and performed the experiments, constructed the plasmids and strains, and wrote the paper.

Ref. II – I performed the library screen and *in vitro* experiments, and participated in the writing of the manuscript.

Ref. III – I planned and performed the experiments and wrote the paper.

ABBREVIATIONS

ABC transporter	ATP-binding cassette transporter
DDE motif	triplet amino acid motif: aspartic acid, aspartic acid, and glutamic acid
Fis	Factor for Inversion Stimulation
IHF	Integration Host Factor
IUPAC	International Union of Pure and Applied Chemistry nomenclature: D – G, A or T nucleotide M – A or C nucleotide (a**M**ino) W – A or T nucleotide (**W**eak interaction-2 H bonds) Y – C or T nucleotide (p**Y**rimidine)
IS element	insertion sequence element
H-NS	Histone-like Nucleoid Structuring Protein
HTH motif	helix-turn-helix motif
HU	Heat-Unstable Nucleoid Protein
LPS	Lipopolysaccharide
OMP	Outer Membrane Protein
Phe^+ colony	Phenol utilising bacterial colony

INTRODUCTION

All living organisms adjust their metabolism and behaviour in response to the changes in the environment. The ability to adapt is a fundamental property of life and constitutes a basic difference between living and nonliving matter. Bacteria are unicellular organisms constantly monitoring a variety of environmental and intracellular parameters because their survival directly depends on the accurate responses to harsh environmental conditions. Adjustment to environmental conditions can be either physiological or genetic. Physiological adaptation takes place during the lifetime of an organism through the changes in the protein functioning or stability, gene expression, *etc*. Two-component signal transduction systems consisting of membrane sensor and cytoplasmic regulator are the most common tools in bacteria to regulate gene expression according to changing environmental conditions. Genetic adaptation, sometimes called also evolutionary adaptation, occurs through the changes in genome and thus is maintained in a population over many generations. Genomic changes are the result of spontaneous mutation or recombination including transposition of mobile elements.

Pseudomonas genus is extraordinary because its excellent ability to adapt to different conditions. Representatives of this family are found in soil and water or colonising different organisms such as plants, animals and humans. Wide ecological niche is gained through the complex signalling systems correlating with the genome size of *Pseudomonas* bacteria and the range of environmental challenges encountered by them. ColR-ColS two-component signal transduction system conserved in *Pseudomonas* genus was first identified due to its importance in root colonising ability of *P. fluorescens* (Dekkers *et al.*, 1998). Following, ColR-ColS system has been implicated in various processes in different *Pseudomonas* species. Our research group has detected the involvement of *P. putida* ColR-ColS system in phenol tolerance and transposition of Tn*4652* under phenol selection conditions (Hõrak *et al.*, 2004). Additionally, we have noticed that ColR-ColS pathway is mediating the adjustment of bacteria to the conditions of Mg^{2+} limitation and preventing the cell lysis on glucose (Putrinš *et al.*, 2008; Teesalu, 2008). Furthermore, ColR-ColS signal transduction has been implicated in the virulence of human pathogen *P. aeruginosa* (Garvis *et al.*, 2009) and in the resistance to heavy metals and some antibiotics (De Weert *et al.*, 2004; Hu and Zhao, 2007). However, the exact function of ColR-ColS signal system has remained unclear and therefore it is hard to understand the associations between different processes affected by this system. Thus, the main objective of my thesis was to understand the role of ColR-ColS signal system in *Pseudomonas* bacteria. As there were some hints about involvement of ColR-ColS pathway in target site selection of Tn*4652* in *P. putida* (Hõrak *et al.*, 2004), I aimed first to clarify the association between ColR-ColS system and transposition of Tn*4652*. For that reason I created a new selection free transposition assay for Tn*4652* and analysed the target site selection of this element. This work disclosed that the effect of ColR-ColS system on transposition is condi-

tional and associated with lowered phenol tolerance of bacteria. In the second part of my thesis I concentrated to revealing the target genes of response regulator ColR in *P. putida*. Several target genes of ColR were identified and, in addition, the putative regulon of ColR-ColS pathway was predicted computationally. Most of the ColR target genes appear to be involved in membrane functioning as the regulon contains several genes coding for membrane proteins as well as genes involved in the homeostasis of lipids, peptidoglycan layer and exopolysaccharides. Thus, the ColR-ColS signal pathway can be considered as the sentinel of bacterial cell envelope.

1. REVIEW OF LITERATURE

Bacteria are microscopic unicellular organisms able to colonise the whole world. Their universal habitats are water, soil, tissues of plants and animals. But they can also cope with conditions as extreme as the acid of volcanic hot springs (Herrera and Cockell, 2007) and the pressure of deep-sea hydrothermal vents (Nichols *et al.*, 2005). Actually, bacteria can tolerate all kinds of extremes of temperature, salinity, heavy metals, toxic compounds, *etc*. Thus, there exists vast diversity among bacteria according to their current residence. Additionally, conditions of a natural habitat can change very quickly and microbes themselves need to be versatile in order to adjust in time. Bacterial adaptation to environmental changes and other stresses is usually mediated by changes in gene expression but also genetic reorganisations favouring adaptation can occur under harsh selection conditions.

I. Genus *Pseudomonas*

Genus *Pseudomonas*, which belongs to Gammaproteobacteria, is one of the most ubiquitous group of bacteria in the world, yet, there is also extraordinary phenotypic and genetic diversity in the group. Initially, all Gram-negative, aerobic rod shaped bacteria that are motile due to polar flagella and do not form spores were classified among *Pseudomonas* (Peix *et al.*, 2009). These bacteria engage in important metabolic activities in the environment, including element cycling and the degradation of biogenic and xenobiotic pollutants (Kertesz and Mirleau, 2004; Timmis, 2002). *P. aeruginosa* is the type species of the genus since it was the first one described by Migula at 1894 (Peix *et al.*, 2009). Following, exceptional nutritional diversity was attributed to the genus. *Pseudomonas* species are very versatile in using different chemical compounds as carbon, nitrogen, and energy source, they can degrade aromatic compounds, halogenated derivatives and recalcitrant organic residues (Spiers *et al.*, 2000). Yet, they tolerate rather limited range of temperatures and hydrogen ion concentrations as there are no true thermophiles or acidophiles in the genus (Palleroni, 2010). After the first description of the genus, there have been several reclassifications as ribosomal 16S RNA sequence-based grouping was introduced. Currently, there are 128 species accepted within the genus (Anzai *et al.*, 2000), among them 17 completely sequenced genomes available at homepage (www.pseudomonas.com). Average GC content of *Pseudomonas* is 61–70% and genome size varies between species from 4.6 Mb-long of *P. stutzeri* to 7.1 Mb of *P. fluorescens* and there are differences even within species. For example, the genome of *P. aeruginosa* spans from 5 Mb to 7 Mb (Schmidt *et al.*, 1996). Large genome size presumably reflects that these bacteria encounter range of ecological niches on regular basis (Spiers *et al.*, 2000) otherwise the size had been reduced to decrease the metabolic and energetic load. In fact the genetic complexity of *Pseudomonas* bacteria approaches that of the simple

eukaryote *Saccharomyces cerevisiae* (cmr.jcvi.org), whose genome encodes about 6200 proteins similarly to *P. fluorescens*. Most of *Pseudomonas* species are environmental bacteria found in soil and water related environments, but there are also species colonising plants, animals and humans. It appears that *Pseudomonas* bacteria do not possess any notable features that would make the genus more evolvable than other genera except they have extensive collection of regulatory genes (Spiers *et al.*, 2000). This could give an advantage in using novel ecological opportunities and niche colonisation. Great ecological and metabolic diversity allows clustering to seven subgroups (Anzai *et al.*, 2000) of which *P. aeruginosa*, *P. putida*, *P. fluorescens* and *P. syringae* are fluorescent, whereas *P. stutzeri*, *P. alcaligenes*, *P. mendocina* are non-fluorescent pseudomonads (Palleroni, 2010). Five representative species of the genus (*P. aeruginosa*, *P. fuorescens*, *P. putida*, *P. syringae* and *P. entomophila*) whose genome structure has been studied have 2065 genes in common (Vodovar *et al.*, 2006). Roughly it means that less than 40% of the proteome is shared among five species, leaving quite an amount of proteins unique for each species. Most investigated species of the genus are the clinically important human pathogen *P. aeruginosa*, the agriculturally important plant pathogen *P. syringae*, the plant growth promoting *P. fluorescens* and the nonpathogenic bioremediation agent *P. putida*.

Pseudomonas aeruginosa is ubiquitous environmental bacterium that grows in soil, marshes and coastal marine habitats, as well as on plant and animal tissues. *P. aeruginosa* is one of the major causes of opportunistic human infections and its prominence as a pathogen is due to its intrinsic resistance to antibiotics and disinfectants (Hancock, 1998; Schweizer, 2003). *P. aeruginosa* is the best described bacterium with regards to quorum sensing (Bjarnsholt *et al.*, 2010), *in vitro* biofilm formation (Schobert and Tielen, 2010) and the development of antibiotic tolerance (Hoiby *et al.*, 2010). Cells of biofilm are more tolerant to antibiotics and therefore thought to be the underlying cause of many chronic infections, including those in the burn wounds and in the lungs of patients with cystic fibrosis (Costerton *et al.*, 1999).

Pseudomonas syringae was isolated from a diseased lilac tree and thus first studied for its ability to cause disease on plants. Although, there are at least 50 pathovars with different pathogenicity and host-range named as *P. syringae*, strain DC3000 became a model for the study of bacterial-plant interactions since it was the first strain sequenced to completion and is pathogen of tomato and *Arabidopsis* (Buell *et al.*, 2003). In addition to disease, the bacterium can be found in association with healthy leaves growing as an epiphyte. Although *P. syringae* interacts with a wide range of plants around the world most of its strains exhibit rather narrow host ranges (Hirano and Upper, 2000). Actually, *P. syringae* has two ways to damage its host – by lesion formation and frost injury. The latter is an interesting phenomenon accompanying with *P. syringae* infection due to its ability to nucleate super cooled water to form ice (Hirano and Upper, 2000). It is notable that the Ice^- *P. syringae* was the first recombinant organism deliberately introduced into the environment.

Pseudomonas fluorescens is plant beneficial or biocontrol microorganism that inhabits the rhizosphere of many plants (Paulsen *et al.*, 2005). The species has important applications in biotechnology because it can enhance plant growth and protect crops against disease. Biocontrol activity of *P. fluorescens* depends mostly on the synthesis of extracellular antimicrobial secondary metabolites and exoenzymes (Haas *et al.*, 2002). These exoproducts are thought to antagonise the pathogenicity of a variety of phytopathogenic fungi. Additionally, critical factor for plant beneficial interaction is the aggressive colonisation ability of bacterium which prevents the invasion of detrimental soil microorganisms onto the root surface (O'Sullivan and O'Gara, 1992). *P. fluorescens* is diverse in catabolic capabilities and able to use various iron siderophores, possesses an extensive set of transport genes and detoxification systems which all together makes it suitable for commensal lifestyle on plants (Paulsen *et al.*, 2005).

Pseudomonas putida was distinguished from plant growth promoting rhizobacterium *P. fluorescens* in determinative tests by its lack of proteolytic activity (Palleroni, 1984). Actually, *P. putida* lacks most of virulence determinants of pathogenic relatives that mediate host damage, for instance, exotoxins, hydrolytic enzymes, type III secretion systems, *etc*. (Nelson *et al.*, 2002) and instead is a saprophytic environmental bacterium found in soil, water and plants (Espinosa-Urgel *et al.*, 2002; Timmis, 2002). First sequenced *P. putida* strain KT2440 (Nelson *et al.*, 2002) was also the first Gram-negative soil bacterium certified as a safety strain by the Recombinant DNA Advisory Committee (Federal Register, 1982) and thus has become the model for the study of *P. putida* (Regenhardt *et al.*, 2002). KT2440 genome is 6.2 Mb long with GC content of 61.5% and contains about 5500 genes (www.pseudomonas.com). Role category has been assigned to 70% of genes leaving up to 30% of genes hypothetical (cmr.jcvi.org). Most of those 3850 genes with assigned role category in strain KT2440 belong to the groups of transport and binding proteins (17%), regulatory functions (14%), energy metabolism (13%), cellular processes (10%) and cell envelope (9%) (cmr.jcvi.org). Despite the obvious lack of virulence determinants, the genome sequence of *P. putida* provides insights into the basis of versatility of this bacterium (Wu *et al.*, 2010). Consistent with its large genome size and flexible environmental adaptation, *P. putida* contains high proportion of regulatory genes and a large number of genes involved in the catabolism, transport and efflux of organic compounds as well as complex repertoire of chemosensory systems and enormous capacity to tolerate heavy metals (Canovas *et al.*, 2003; Nelson *et al.*, 2002). The size and complexity of the *P. putida* genome probably reflects an evolutionary adaptation permitting it to thrive in diverse environments and resist the effects of a variety of conditions (Spiers *et al.*, 2000; Wu *et al.*, 2010). Additionally, *P. putida* is genetically easily manipulated and therefore a convenient model organism for experimental genetics (Bechthold, 2005; Ramos *et al.*, 1987). All these characteristics of *P. putida* make this bacterium an important subject of study and the accumulating knowledge has considerable potential for new

biotechnological applications in agriculture, biocatalysis, bioremediation and bioplastic production (Gosset, 2009; Kagle *et al.*, 2009).

II. Physiological adaptation of bacteria

Physiological or environmental adaptation of bacteria is achieved through complex regulatory networks that constantly monitor extracellular conditions and regulate cellular physiology according to rapidly changing environment. There are different regulatory circuits used by bacteria; from single component systems to multi-component relays and regulatory cascades (Hoch, 2000; Ulrich *et al.*, 2005; West and Stock, 2001), which include various components such as kinases and phosphatases, adenylate and diguanylate cyclases, phosphodiesterases, response regulators, chemotaxis proteins, *etc*. (Galperin, 2004). Furthermore, bacterial cells communicate with each other and sometimes exhibit coordinated behaviour similar to that of multi-cellular organisms. This behaviour is governed by intercellular signalling including, for example, well-studied quorum sensing mechanism, which allows detecting the density of a population (Atkinson and Williams, 2009; Duan *et al.*, 2009). Regulatory pathways can also be divided according to the magnitude of their function, some regulators affect very many cellular targets and are called global (Baker *et al.*, 2006; Magnusson *et al.*, 2005; Won *et al.*, 2009), whereas the others regulate only very specific processes or genes.

Two-component systems are the dominant type of signal transduction in prokaryotes to monitor critical parameters of the external environment and internal physiology and inform the cellular transcriptional machinery or other targets about the actual changes. Conserved mechanism of information propagation used by these proteins is found on the phosphoryl group transfer. The overview of bacterial two-component system proteins structure and function is given in the next chapters of the thesis.

Bacterial two-component signal transduction systems

Schematic overview of bacterial two-component signal system is presented in Figure 1. Classically bacterial two-component pathways consist of a sensor protein located in the cytoplasmic membrane and a response protein located in the cytoplasm (Gross *et al.*, 1989). Extracellular or periplasmic domain of the sensor detects the signal, which could be an effector molecule or change in some other parameter and autophosphorylates at a conserved cytoplasmic histidine residue (Dutta *et al.*, 1999). Thus, most of two-component sensors are histidine kinases and the phosphate is subsequently transferred to an aspartate residue of the cognate response regulator protein (Stock *et al.*, 1989). Phosphorylated regulator will become active and then modifies cell physiology according to sensed signal. Most of prokaryotic response regulators are transcription

factors and activate or repress the transcription of its target genes (Robinson *et al.*, 2000; Rodrigue *et al.*, 2000; Stock and Da Re, 2000).

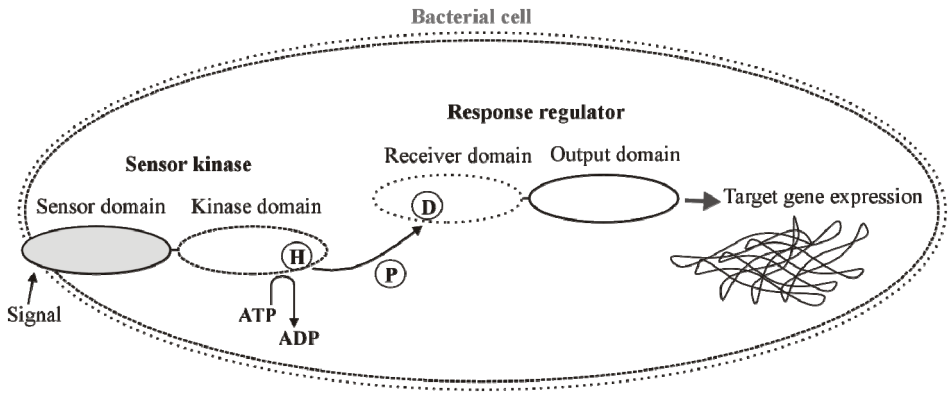

Figure 1. Schematic representation of two-component signal transduction system. Extracellular signal activates sensor kinase, which autophosphorylates at a conserved histidine residue (H) using ATP molecule as a phosphate (P) donor. Following, response regulator initiates the phosphotransfer to its conserved aspartate (D), undergoes activation and affects target gene expression.

The first two-component pathway characterised was nitrogen responsive NR system in *E. coli* (Ninfa and Magasanik, 1986). Thereafter, many two-component pathways involved in different processes have been described in various bacteria, but it has also been acknowledged that their distribution is not uniform among prokaryotes. There is obvious correlation between the habitat and the number of regulatory proteins in bacterium. For example, *Pseudomonas fluorescens* has 82 genes for histidine kinases and 120 genes for response regulators and it has to endure array of fluctuating conditions as plant commensal bacterium (Paulsen *et al.*, 2005). On the other hand, human pathogen *Mycoplasma genitalium*, which is the simplest organism capable of independent life with a minimal set of genes, encodes no components of two-component pathways (Fraser *et al.*, 1995). This could be explained with *M. genitalium* parasitic lifestyle in a relatively constant environment of its host organism. Two-component systems are also found in several eukaryotes (Chang and Stewart, 1998; Mizuno, 2005), but not in mammals and therefore have gained a lot of interest as potential antimicrobial targets (Fernandez *et al.*, 2010; Otto, 2009; Stephenson and Hoch, 2004; Watanabe *et al.*, 2008).

Sensor kinases

Typical bacterial sensor histidine kinases are homodimeric integral membrane proteins consisting of N-terminal extracellular or periplasmic input domain and C-terminal cytoplasmic transmitter domain. Prototype of sensor kinases is *Escherichia coli* osmosensor EnvZ (Mascher *et al.*, 2006). It has been demonstrated that the number of EnvZ dimers in a cell is about 50 in rich medium in exponential phase of growth (Cai and Inouye, 2002).

Sequences of sensor kinases are more variable than those of the response regulator proteins, especially when the input domain is considered (Cheung and Hendrickson, 2010; Mascher *et al.*, 2006). There are three types of sensor domains based on the structure and cellular location (Cheung and Hendrickson, 2010). In addition to the most common extracellular input domain, there are several examples of membrane embedded sensor domains like the one of thermosensitive histidine kinase DesK of *Bacillus subtilis* (Albanesi *et al.*, 2009; Martin *et al.*, 2009), and proteins with cytoplasmic sensor domains like the oxygen sensing FixL of *Rhizobia* species (Gong *et al.*, 1998; Gong *et al.*, 2000). Different sensory domains are needed to recognise different signals, which can be chemical compounds, changes in membrane potential or other physical parameters. Signal is mostly the least understood step in a two-component pathway and the exact changing factor detected remains vague even for the best described examples of pH and osmosensors (Galperin, 2004). Osmolarity of the medium affects many aspects in the cell physiology and therefore it is difficult to understand, which parameter is the real signal. Yet, there are also some examples where the binding of a compound to a sensor has been demonstrated. Sensor of TodS-TodT pathway regulating toluene degradation binds toluene molecule and with lower affinity also benzene and styrene (Busch *et al.*, 2007; Lacal *et al.*, 2006). Ubiquinone-binding site was detected on the periplasmic loop of global redox sensor RegB in *Rhodobacter capsulatus* (Swem *et al.*, 2006) and LovK sensor detecting visible light has been demonstrated to bind flavin cofactor in *Caulobacter crescentus* (Purcell *et al.*, 2007). Sensor of PhoP-PhoQ two-component regulatory system governing several virulence traits in *Salmonella typhimurium* can bind both Mg^{2+} and Ca^{2+} cations (Vescovi *et al.*, 1997). In addition to smaller molecules, some sensor histidine kinases have periplasmic partners that first bind a ligand and then activate sensor kinase upon binding (Falke *et al.*, 1997). For example, chemosensory pathway enabling bacterial cells to sense and swim up or down gradients of several chemical attractants and repellents uses monomeric soluble receptors for maltose, galactose, glucose, ribose and dipeptides to mediate this kind of multi-stimulus sensing (Falke *et al.*, 1997). Recently, it has been demonstrated that accessory proteins can also influence the active state of a sensor, as the examples of membrane proteins that modulate the kinase or phosphatase activity of sensor have started to accumulate (Eguchi *et al.*, 2007; Gerken *et al.*, 2009; Goodman *et al.*, 2009).

How the signal is transmitted across the membrane from sensory to cytoplasmic domain is still not completely understood, but most probably it occurs through conformational changes of a protein. It is suggested that dimerisation of a sensor could be necessary for signal transduction (Galperin, 2004).

Cytoplasmic kinase core of sensor protein is much more understood due to a more conserved sequence and structure, which can be further divided into independent dimerisation domain and catalytic domain (Tomomori *et al.*, 1999). Kinase core is responsible for ATP binding and trans-autophosphorylation at a conserved histidine residue (Tanaka *et al.*, 1998). This means that one monomer within the histidine kinase dimer phosphorylates the other with a γ-phosphate from ATP. Catalytic motif of histidine kinase is unique and thus defines independent family of protein kinases, but the ATP binding fold has some similarities to the ATPase family whose members are also DNA gyrase and mismatch repair protein MutL (Khorchid and Ikura, 2006; Robinson *et al.*, 2000). Autophosphorylation of a sensor protein is followed by the transfer of the phosphate to a partner response protein or dephosphorylation. Interaction between the transmitter domain of a sensor and a cognate receiver domain is specific and therefore the occasions of cross-talk between the non-cognate pairs are very rare (Fisher *et al.*, 1996; Skerker *et al.*, 2005; Yamamoto *et al.*, 2005). Sensor kinases have also the ability to act as phosphatases and dephosphorylate its partner response regulator (Igo *et al.*, 1989; Jin and Inouye, 1993; Zhu *et al.*, 2000).

Response regulators

Typical response regulator consists of an N-terminal receiver or regulatory domain connected to a C-terminal output or effector domain. Usually, the genes encoding a two-component pathway are in the same operon, but the amount of response regulator exceeds by far that of a sensor protein. This is gained through the differential regulation at the level of translation initiation (Berman and Jackson, 1984; Cai and Inouye, 2002; Comeau *et al.*, 1985). For example, there is about 30-fold excess of response regulator monomers over the sensor ones in case of EnvZ-OmpR signal pathway in the cell of *E. coli* (Cai and Inouye, 2002).

Receiver domains of different response regulator proteins are well-conserved and can be defined on the bases of sequence similarities. There are over thousands of protein domains assigned as response regulator receiver, but only few of them have been studied experimentally (Bourret, 2010). CheY protein found in several enteric species has become the structural prototype for the receiver domains of two-component response regulators (Stock *et al.*, 1989; Volz and Matsumura, 1991). CheY is a single-domain protein activated by phosphorylation induced conformational change and interacts with flagellar components to regulate bacterial motility systems to ensure the accurate chemotaxis (Jenal and Galperin, 2009). Actually, about 15% of all response regulators

in bacteria consist only of a receiver or so called phosphoacceptor domain and thus function as stand-alone module (Bourret, 2010). These proteins can regulate different functions despite their high similarity. Another example of such protein is *Bacillus subtilis* Spo0F receiver domain, which participates in the sporulation controlling phosphorelay as a phosphotransfer protein (Varughese, 2002). The active site of the receiver domain is a small acidic pocket comprising of five residues that are crucial for phosphotransfer and are termed catalytic residues. In case of CheY protein, the active site consists of a phosphorylated aspartate, two additional aspartates involved in coordination of a Mg^{2+} ion that is essential for phosphorylation, and lysine and threonine involved in the phosphorylation-induced conformational change (Appleby and Bourret, 1998; Sanders *et al.*, 1989). Active site catalyses the phosphotransfer from sensor kinase protein to a conserved aspartate located at the bottom of the acidic pocket. Receiver domains are also capable of autophosphorylation in the presence of small molecule phosphodonors (Lukat *et al.*, 1992). Phosphorylation of a receiver domain induces a conformational change that results in the activation of an associated output domain that executes the response (Anand *et al.*, 2000; Hwang *et al.*, 1999).

Bacterial two-component output domains are extremely diverse and can exist in various combinations. There are over 40 different families of output domains according to their structures, whereas the two-thirds of all response regulators carry different DNA-binding domains and thus serve as transcriptional factors (Galperin, 2006, 2010). The remaining third of response regulators, that do not bind DNA, can be categorised by their output domain as RNA-binding, protein-binding or enzymatic (Galperin, 2006). RNA-binding response regulators usually act as transcription anti-terminators (Shu and Zhulin, 2002), whereas protein phosphatase and phosphodiesterase domains are the most common output domains among those with the enzymatic activity (Galperin, 2006). The variety of output domains includes membrane transporters and methylesterases (Falke *et al.*, 1997; Galperin, 2010), domains participating directly in the control of protein stability or in the modulation of the level of cyclic nucleotides, *etc*. (Bouche *et al.*, 1998; Pratt and Silhavy, 1996; Thomason *et al.*, 1998; Wassmann *et al.*, 2007). Obviously, there is also a significant fraction of output domains whose functions have remained unknown.

Response regulators that act as transcription factors usually have a version of helix-turn-helix DNA binding motif, although there are also exceptional structures consisting mostly of β-sheets (Sidote *et al.*, 2008). The response regulators with helix-turn-helix motif are further classified to subgroups according to the slight differences in their protein structure. Three subfamilies, OmpR/PhoB, NtrC/DctD, and NarL/FixJ, are by far the most wide-spread of DNA-binding HTH output domains in bacteria (Mizuno, 1997). The OmpR/PhoB subfamily of response regulators recognises a direct repeat sequence in the promoter region of their target genes (Kenney, 2002). For example, *B. subtilis* response regulator YclJ recognises a direct repeat of the consensus sequence TTCATANTTT and phosphate homeostasis regulator PhoB binds to

sequence TGTCA appearing twice in every *pho* box (Kenney, 2002; Ogura *et al.*, 2010). Several other response regulator subfamilies bind to palindromic sequences, for example, the binding consensus of global regulator RegA contains a motif of two GCGNC inverted repeats (Laguri *et al.*, 2003). It is usual for unphosphorylated response regulators to exist as monomers in the solution, although there are exceptions like the members of NtrC family that form dimers (Galperin, 2010). Phosphorylation of the receiver domain induces the dimerisation, which plays a key role in the regulation of DNA-binding response regulators, dramatically improving their binding to the repeat sequences (Birck *et al.*, 2003; Martinez-Hackert and Stock, 1997; Robinson *et al.*, 2000). For example, phosphorylation of the osmoregulator OmpR increases its DNA binding activity 10-to-30-fold (Head *et al.*, 1998). However it is important to point out that the phosphorylated OmpR exists as a monomer in the solution and the protein dimerises only on DNA (Rhee *et al.*, 2008; Yoshida *et al.*, 2006). It has been proposed that OmpR molecule first binds to DNA and then undergoes a conformational change that promotes the phosphorylation of the N-terminal receiver domain, which induces the dimerisation of the receiver domains and that causes the binding of a second monomer to DNA (Rhee *et al.*, 2008). Phosphorylation of a regulator can induce different multimers, but the active form is mostly dimer and all the other complexes are inactive (Galperin, 2010; Paul *et al.*, 2007). Response regulator proteins also have the ability for reversed reaction and thus act as autophosphatases (Bourret, 2010; West and Stock, 2001) or have accessory proteins that have the ability to induce their autophosphatase activity (Pazy *et al.*, 2009; Zhao *et al.*, 2002). Typically, in addition to regulating the expression of target genes two-component response regulators bind to the DNA of their own promoter and execute auto-regulation (Bijlsma and Groisman, 2003; Deng *et al.*, 2010).

Examples of some well-known two-component systems

Phosphorylation-based signal transduction of bacterial two-component pathways is quite well characterised, but the molecular basis of the role of two-component systems in bacterial physiology and adaptation has remained quite vague. Mostly, some processes regulated by a system are known but the real target genes affected have not been identified or *vice versa*. Two-component systems have been demonstrated to regulate different processes like DNA replication and cell division (Jenal, 2000), extracellular communication (Lopez and Kolter, 2010), sporulation (Hoch, 2000; Sonenshein, 2000), chemotaxis (Falke *et al.*, 1997), metabolism (Qian *et al.*, 2010), transport (Choi *et al.*, 2009), osmoregulation (Mizuno and Mizushima, 1990), stress response (Dorel *et al.*, 2006), antibiotic resistance (Fernandez *et al.*, 2010; Hong *et al.*, 2008), virulence (Cheung *et al.*, 2010; Rood, 1998), *etc*.

E. coli two-component pathway EnvZ-OmpR is one of the most studied systems and therefore its sensor protein EnvZ has become the structural

prototype for sensors and the response protein OmpR has become the archetype example of the largest subfamily of DNA binding response regulators (Igo and Silhavy, 1988; Pratt *et al.*, 1996; Yoshida *et al.*, 2006). The main function of this system is to regulate the levels of major porins as well as other outer membrane proteins (OMP) according to the fluctuations in the osmolarity of the medium (Forst *et al.*, 1989). *Escherichia coli* has two major outer membrane porins, OmpF and OmpC, which serve as passive diffusion pores across the outer membrane (Nikaido *et al.*, 1983). At low osmolarity, the larger pore OmpF is expressed preferentially, whereas at high osmolarity, the narrower pore OmpC dominates (Pratt *et al.*, 1996). This reciprocal regulation of porin genes is possible due to the presence of four and three OmpR binding sites in the promoters of *ompF* and *ompC*, respectively. These 20-basepair DNA regions contain two tandemly arranged 10-bp half-sites each of which binds phosphorylated OmpR protein allowing the OmpR dimer to form on the DNA (Harlocker *et al.*, 1995). GTTACATATT is the 10-basepair sequence with the highest affinity for OmpR and it is in the promoter of *ompF*, whereas the underlined G nucleotide and ACA sequence were demonstrated to be important for OmpR binding (Yoshida *et al.*, 2006). Expression of the *ompF* gene is achieved by the hierarchical occupation of three adjacent strong binding sites by phosporylated OmpR. The binding sites in the promoter of *ompC* are with weaker affinity and therefore the expression of OmpC is achieved only when the amount of phosphorylated OmpR increases in the cell. Rich medium induces the kinase activity of a sensor EnvZ compared to that of its phosphatase activity and thus high osmolarity increases the amount of phosphorylated OmpR (Jin and Inouye, 1993). At the same time, the forth binding site in the promoter of *ompF*, which is also the low affinity site, becomes occupied by OmpR and forms a loop with OmpR molecules bound to the high affinity sites and this represses the transcription from *ompF* (Bergstrom *et al.*, 1998). Taken together, EnvZ-OmpR signal pathway conducts an elaborate regulation of porin genes and it has been suggested that the larger pore size of OmpF is important for the efficient nutrient uptake from nutritionally poor medium while the smaller pore size of OmpC could be important to exclude the passage of toxic bile salts in the gut (Ferrario *et al.*, 1995). Despite the clear indications of high osmolarity as the condition activating this pathway, the real input signal recognised by EnvZ sensor has remained a mystery.

It has become apparent that in addition to regulating the levels of outer membrane porins, EnvZ-OmpR signal pathway is involved in the regulation of many cellular responses in different bacteria such as *E. coli*, *Salmonella enterica, Yersinia,* and *Shigella*. Regulator OmpR plays a central role in controlling the expression of both housekeeping and virulence genes influencing processes such as flagella assembly, biofilm formation, pathogenesis and many more (Dorman *et al.*, 1989; Park and Forst, 2006; Shin and Park, 1995; Vidal *et al.*, 1998). Actually, EnvZ-OmpR is one of the most important signal pathways in *E. coli* as its deletion has the second most dramatic phenotype among the deletions of all two-component systems in this organism (Cowan *et al.*, 1992). The

regulon of OmpR contains over 125 genes, including porin genes *ompF* and *ompC*, *flhDC* operon controlling the expression of flagella regulon, a subset of regulatory small RNAs, genes for type III secretion systems, other two-component systems *etc*. (Brzostek *et al.*, 2007; Guillier and Gottesman, 2006; Lee *et al.*, 2000; Oshima *et al.*, 2002; Shin and Park, 1995).

Another, well-characterised example of two-component signal system in enteric bacteria is PhoP-PhoQ consisting of the Q protein which is a sensor for extracytoplasmic divalent cations and its cognate response regulator protein P (Groisman, 2001). Although studied in different species like *E. coli*, *B. subtilis*; *P. aeruginosa*, *etc*., most of the work with PhoP-PhoQ has been carried out with human pathogen *Salmonella enterica* (Ernst *et al.*, 2001; Groisman, 2001). Three different cues have been demonstrated to activate the PhoP-PhoQ system: a mild acidic pH, antimicrobial peptides, and low Mg^{2+}. At first, extracellular or periplasmic Mg^{2+} concentration was thought to be the primary signal for PhoP-PhoQ pathway (Vescovi *et al.*, 1997). In addition to Mg^{2+}, divalent Ca^{2+} and Mn^{2+} cations were demonstrated to bind sensor PhoQ (Garcia Vescovi *et al.*, 1996). Binding of cations stabilises the conformation of PhoQ that is unfavourable for the activation of the PhoP and leads to the repression of PhoP-regulated genes (Groisman, 2001). Thus, low concentration of cations activates this system. Now, it is known that cationic antimicrobial peptides compete with divalent cations for binding to PhoQ, whereas their binding initiates signal cascade which leads to the activation of PhoP-controlled genes (Bader *et al.*, 2005). Additionally, at first mild acidic pH was thought to affect only a subset of PhoP-regulated genes independent of the sensor PhoQ (Soncini and Groisman, 1996), but now it has been shown that PhoQ is directly activated by acidic pH as the periplasmic domain adopts a different conformation when cation-formed stabilising bonds are interrupted in the acidic environment (Prost *et al.*, 2007). All these signals interpreted by PhoP-PhoQ system are found in the phagosome environment and thus allow *Salmonella* to activate protection programme to avoid killing within these cells. It is important to keep in mind that while *Salmonella* is facultative intracellular pathogen, conserved PhoP-PhoQ pathway can be found in species with different lifestyle and thus could be mediating cues other than intracellular environment (Gooderham and Hancock, 2009; Monsieurs *et al.*, 2005; Perez *et al.*, 2009).

PhoP-PhoQ regulatory system governs several virulence traits in different species (Cheng *et al.*, 2010; Garcia Vescovi *et al.*, 1994; Gooderham and Hancock, 2009; Miller *et al.*, 1989; Tzeng *et al.*, 2006), mediates adaptation to low Mg^{2+} environments (Smith *et al.*, 1998; Soncini and Groisman, 1996) and resistance to antimicrobial peptides (Barrow and Kwon, 2009; Moon and Gottesman, 2009; Nakka *et al.*, 2010), regulates bacterial envelope modification (Moon and Gottesman, 2009; Murata *et al.*, 2007; Newcombe *et al.*, 2005) and many other cellular processes. Response regulator PhoP affects the expression of numerous genes, for example in *Salmonella typhimurium* the regulon of PhoP-PhoQ contains over 100 genes (Kato and Groisman, 2008). These genes encode Mg^{2+} transporters, enzymes involved in the modification of cell enve-

lope components, different virulence proteins and many more including other two-component regulatory systems RstA-RstB and PmrA-PmrB and small RNAs (Kato and Groisman, 2008; Lejona *et al.*, 2003; Overgaard *et al.*, 2009; Wösten *et al.*, 2000). Some of these genes are directly regulated by PhoP binding to their promoter-element. Conserved motif recognised by this transcription factor is a heptanucleotide direct repeat sequence (G/T)GTTTA(A/T) (Lejona *et al.*, 2003). Other genes are influenced indirectly via other regulatory proteins whose expression is subject to PhoP-mediated control (Kato and Groisman, 2008). Although similar processes are regulated by PhoP-PhoQ system in several species, it is now established that the majority of targets governed by the regulator PhoP are not shared across species (Perez *et al.*, 2009) and even a single PhoP-PhoQ pathway can initiate the regulation of different subsets of target genes according to the varying input signals (Choi *et al.*, 2009).

Best described two-component system in *Pseudomonas* family consists of sensor GacS and response regulator GacA. Although found in many gamma-proteobacteria, this pathway has gained a lot of attention due to its role in the chronic persistent infection of *P. aeruginosa* (Goodman *et al.*, 2004). Sensor kinase GacS was first identified as a pathogenicity regulator in *P. syringae* (Hrabak and Willis, 1992) and response regulator GacA as a global regulator of secondary metabolites in *P. fluorescens* (Laville *et al.*, 1992). Later GacA also has been named as the master regulator of virulence (Rahme *et al.*, 2000). Although known by different names such as *S. enterica* BarA-SirA, *Erwinia carotovora* ExpS-ExpA, *Vibrio cholerae* BarA-VarA, *E. coli* BarA-UvrY and GacA-GacS in *Pseudomonas* family, this pathway has a similar role in the pathogenicity of these species (Tomenius *et al.*, 2006; Vallet-Gely *et al.*, 2010) and conserved mechanism of action (Lapouge *et al.*, 2007; Lapouge *et al.*, 2008). GacA-GacS signal pathway regulates utilisation of growth substrates, lipopolysaccharide composition and resistance to detergents, motility, luminescence and symbiotic squid colonisation in *V. fischeri* (Whistler and Ruby, 2003; Whistler *et al.*, 2007), type III secretion and extracellular plant cell-wall degrading enzymes in *E. carotovora* (Lebeau *et al.*, 2008), quorum sensing machinery, synthesis of extracellular products and biofilm formation in *P. aeruginosa* (Goodman *et al.*, 2009; Kay *et al.*, 2006), biofilm formation and biosynthesis of storage glycogen in *E. coli* (Wang *et al.*, 2005), production of antibiotic compounds, swarming motility, oxidative stress and biocontrol in *P. fluorescens* (Kay *et al.*, 2005).

The regulatory mechanism of GacA-GacS pathway is interesting since in addition to leading to the regulation of transcription, it can be seen as the regulator of translation initiation. Namely, phosphorylated response regulator GacA promotes transcription of small non-coding RNAs (Heeb and Haas, 2001). These small RNA molecules have stem-loop forming repeat sequences and bind a family of proteins that otherwise act as translational repressors of certain mRNAs. Therefore, GacA-regulated small RNAs sequester translation repressors, hence indirectly initiate translation. However, in addition to sRNA genes the homologues of GacA in *S. enterica* and *Legionella pneumophila* have

been shown to regulate other types of genes encoding flagellar functions and transcription factors (Sahr *et al.*, 2009; Teplitski *et al.*, 2003; Teplitski *et al.*, 2006). The output of GacA-GacS signal cascade or the target genes that are translationally regulated by this system vary considerably among species even though the small RNAs affected are highly conserved and the processes regulated taken together associate to virulence, colonisation and cellular adherence properties (Valverde and Haas, 2008). Little is known about the mode of DNA recognition by response regulator GacA. Conserved palindromic sequence has been found to be necessary for the expression of small RNAs in *P. fluorescens*, *P. aeruginosa* and *L. pneumophila* (Brencic *et al.*, 2009; Humair *et al.*, 2010; Rasis and Segal, 2009). IUPAC consensus TRAGMMWWDDCTYA can be compiled of the demonstrated binding sequences for phosphorylated GacA in the promoters of *rsmY* and *rsmZ* RNAs in *P. aeruginosa* and *L. pneumophila* (Brencic *et al.*, 2009; Rasis and Segal, 2009). However, additional less-conserved sequences appear to be involved in the activation of sRNA genes and it has been suggested that other proteins are needed to facilitate the interactions between GacA protein and RNA polymerase to initiate transcription of small RNA genes (Humair *et al.*, 2010).

Another common thing between GacA-GacS homologues in different species is that the expression of RNA genes controlled by this system increases strongly with increasing cell population densities (Kay *et al.*, 2005; Zuber *et al.*, 2003). This means that the signal molecule activating GacA-GacS pathway is similar to those of quorum sensing autoinducers and mediates social behaviour of the population. Interestingly, the exact signalling molecule is yet to be elucidated but it was demonstrated that the same signal is recognised between different *Pseudomonas* species (Dubuis and Haas, 2007).

ColR-ColS two-component signal system

ColR-ColS pathway is well-conserved between the species of *Pseudomonas* genus (www.pseudomonas.com). When comparing *P. putida* response regulator ColR with those of other family members, the identities start from 99% for *P. entomophila* and end with the 77% of *P. stutzeri*. Interestingly, *P. syringae* has a second copy of a ColR-like protein, although the identity with *P. putida* protein is only 56%. Sensor proteins are less conserved and thus there are slight differences even within *P. putida* strains and the identity with *P. stutzeri* ColS is only 52%. It is possible that ColR-ColS signal pathway is even more wide-spread among bacteria. For example, three ColR-ColS homologues have been found in *Xanthomonas campestris*, although the identity is only 54% and less (Zhang *et al.*, 2008). Conservation of these genes in *Pseudomonas* family indicates to their importance in the fitness of these bacteria, although the exact role of ColR-ColS pathway is still obscure. This signal system has received the attention of scientists studying root colonisation of *P. fluorescens* (de Weert *et al.*, 2006; Dekkers *et al.*, 1998), growth and pathogenicity of *P. aeruginosa* and

Xanthomonas campestris (Duan *et al.*, 2003; Garvis *et al.*, 2009; Zhang *et al.*, 2008) and stress response of *P. putida* (Hu and Zhao, 2007; Hõrak *et al.*, 2004; Putrinš *et al.*, 2008). Despite seemingly unrelated phenotypes occurring in case of ColR deficiency in different species, several parallels can be drawn between the results of different studies. Hence, it is probable that the function of ColR-ColS pathway and the regulated processes are also conserved among different species. Various phenotypes occurring in case of defect ColR-ColS signalling in different bacteria are summarised in Figure 2.

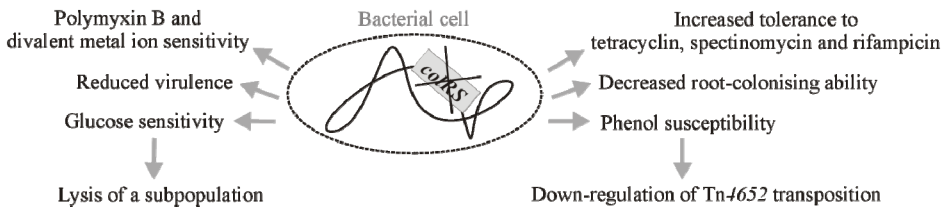

Figure 2. Different phenotypes detected in various *Pseudomonas* species with non-functional ColR-ColS signal system.

ColR-ColS two-component pathway was originally identified as an important component of the root-colonising ability of *P. fluorescens* (Dekkers *et al.*, 1998). Actually, the root colonisation ability of bacteria lacking functional sensor ColS *per se* was not affected, but the cells defective in ColR-ColS signalling demonstrated impaired colonisation in competition with the parent strain on potato, tomato, wheat and radish root tips (Dekkers *et al.*, 1998). Therefore, ColR-ColS system could also be important in bacterial communication and not strictly in colonisation. Similarly, the involvement of ColR-ColS pathway in the communication of bacteria was suggested in *P. aeruginosa* as the expression of *colRS* genes was up-regulated in the presence of two Gram-positive normal human lung resident bacteria *Streptococcus* and *Staphylococcus* (Duan *et al.*, 2003). The activation of *colRS* promoter was also seen when *P. putida* cells were studied in the maize rhizosphere (Ramos-Gonzalez *et al.*, 2005). This means that in addition to bacterial interactions ColR-ColS system could also be important in the communication between a plant and a bacterium. Although, plant exudates were seen to elevate the expression of *colRS* promoter, the exact signal recognised by ColR-ColS pathway has remained obscure (Ramos-Gonzalez *et al.*, 2005).

Following, ColR-ColS two-component system was related to the regulation of transposition of Tn*4652* in *P. putida* (Hõrak *et al.*, 2004). The effect of ColR and ColS on transposition and other mutational processes in plasmid test system (personal communication with R. Hõrak) was registered under phenol starvation conditions and by now it is known that the repression of transposition was due to the increased phenol sensitivity of bacteria lacking proper ColR-ColS system

(Ref. II). Phenol is an aromatic compound that accumulates into the membrane (Sikkema *et al.*, 1994, 1995) and therefore these data indicate that ColR-ColS pathway could be involved in the regulation of membrane functions. Phenol susceptibility of *P. putida* bacteria with dysfunctional ColR-ColS system and its involvement in mutational processes is further discussed in the chapter of Results and Discussion of this thesis.

Soon, ColR-ColS signal system was implicated in the tolerance of several compounds. First, *P. fluorescens* cells with non-functional ColR-ColS signalling were demonstrated to be more sensitive to lipopolysaccharide-binding compound polymyxin B (de Weert *et al.*, 2006). The authors speculated that the polymyxin sensitivity could appear due to alterations in the composition of the outer membrane and demonstrated that ColR-ColS system directly regulates an operon, which is most probably involved in fine tuning of the permeability of the outer membrane (de Weert *et al.*, 2006). Other lab showed that the absence of functional ColR-ColS pathway rendered *P. putida* susceptible to several divalent metal ions suggesting that this signal transduction system is regulating heavy metal resistance, whereas, being especially crucial for the resistance or homeostasis of Mn^{2+} ions (Hu and Zhao, 2007). Somewhat controversially, ColS-deficient cells of *P. fluorescens* were more resistant to several antibiotics like rifampicin, spectinomycin and tetracycline (de Weert *et al.*, 2006). However, these antibiotics do not target the membrane and therefore are not comparable to the cationic antibiotic polymyxin B and Mn^{2+} ions which bind to the membrane. Thus, differences in chemical composition of agents could account for the antagonistic resistance behaviour of *colR* mutant bacteria to different molecules. Taken together, all these results further confirm that bacterial membrane could be altered in cells with mutated ColR-ColS pathway.

Recently, involvement of ColR-ColS signal system in virulence of *P. aeruginosa* (Garvis *et al.*, 2009) and glucose induced cell death of *P. putida* (Putrinš *et al.*, 2008) was observed. Namely, ColR-ColS two-component regulatory system of *P. aeruginosa* was necessary to virulence when measured in *C. elegans* killing assay and to the ability of bacteria to adhere to human epithelial cells (Garvis *et al.*, 2009). This is in good accordance with *P. fluorescens* results where sensor ColS was implicated in the root colonisation (Dekkers *et al.*, 1998). Adherence is an essential step in the process of colonisation, allowing the bacteria to persist, expand in number and establish contact with host cells or other surfaces. On the other hand, in case of *P. putida* dysfunctional ColR-ColS system caused glucose sensitivity of solid-medium-grown cells, which lead to the lysis of a subpopulation (Putrinš *et al.*, 2008). As intriguing as glucose toxicity would be, now it has become evident that the lysis on glucose occurs due to the inability of *colR*-knockout bacteria to tolerate the expression of glucose inducible porin OprB1 and not that glucose itself was killing bacteria (Putrinš *et al.*, 2010a). Too high amount of outer membrane porins in glucose-grown bacteria probably further destabilises the imperfect membrane of ColR-ColS deficient cells until the collapse, which is seen as a lysis of bacteria.

There are many reports about the processes which need participation of ColR-ColS two-component signalling pathway, but very few actual target genes of this system are known. As mentioned above, de Weert *et al.* (2006) revealed that in *P. fluorescens* response regulator ColR regulates an operon, which products are suggested to participate in the composition and permeability of the outer membrane. Namely, the operon called *orf222-inaA/wapQ* locates just downstream of *colRS* genes in the genomes of *Pseudomonas* bacteria (www.pseudomonas.com) and putatively encodes methyltransferase and lipopolysaccharide kinase (de Weert *et al.*, 2006). The same was confirmed for *P. putida* and will be discussed in the Results chapter of this thesis. Here, it is interesting to note that the expression of this ColR-regulated operon was induced in the presence of cell division inhibiting antibiotic ceftazidime in *P. aeruginosa* (Blazquez *et al.*, 2006). In this light, one could hypothesise that ColR-ColS system is regulating some functions involved in cell division. Actually, potential participation of ColR-ColS system in cell division was recently suggested also in *P. putida* as only growing cells with defect ColR-ColS signalling pathway were phenol sensitive, whereas the starving ones were not affected (Putrinš *et al.*, 2010b). Taken together, all the data about ColR-ColS system is indicating to membrane as the main target of this signal transduction pathway and most probably the synthesis or turnover of membrane components could be impaired in bacteria with non-functional ColR-ColS signalling.

III. Genetic adaptation

Permanent adaptation to a new environment or stress needs in addition to flexible regulatory signal transduction network also genetic variability within a bacterial population which allows for the selection of the best adapted genotypes. Genetic variability is achieved trough mutational processes, recombination and gene transfer. Bacteria have highly dynamic genomes, for example, stored *E. coli* strains were shown to accumulate spontaneous genetic variation correlated to time of storage (Naas *et al.*, 1995). This indicates that bacteria under nutritional deprivation accumulate mutations which could be useful for adaptation. Particularly noteworthy, because of their involvement in bacterial evolution and adaptation to challenging environments, are genes belonging to the groups of DNA replication, recombination, modification and repair and those involved in processes such as adaptation, protection, as well as antibiotic resistance. Among other mutational processes transposition is one of the possibilities to create genetic diversity to facilitate adaptation (Kivisaar, 2003). Activation of transposition has been demonstrated for many elements in different stress conditions, for instance, yeast *Saccharomyces cerevisiae* retrotransposons are ethanol stress-induced (Stanley *et al.*, 2010), movement of *Deinococcus radiodurans* element IS*Dra2* was found to be strongly induced by irradiation (Mennecier *et al.*, 2006; Pasternak *et al.*, 2010), IS*10* transposition was shown to be induced by UV light in *E. coli* (Eichenbaum and Livneh, 1998),

transposition of several IS elements is elevated in *Burkholderia multivorans* at a high temperature (Ohtsubo *et al.*, 2005), the movement of insertion sequences of *Burkholderia cenocepacia* is mediated by oxidative stress (Drevinek *et al.*, 2010) and *P. putida* transposon Tn*4652* is mobilised in the starvationary phase of growth (Ilves *et al.*, 2001). Overview of transposition and its regulation in bacteria is given below since this thesis is concentrated onto the adaptation of *P. putida* and the involvement of transposon Tn*4652* in it.

Overview of transposition regulation in bacteria

Transposons are mobile genetic elements able to move to new locations, without the need for sequence homology between transposon and its target site (Hickman *et al.*, 2010). Transposase is a mobile element-encoded enzyme which implements recombination events between the ends of the transposon and the target site. There are several different types of proteins mediating transposition, but the biggest class is DDE transposases named after amino acid triplet (D – aspartic acid, E – glutamic acid) of the active site (Haren *et al.*, 1999; Kennedy *et al.*, 2000; Polard and Chandler, 1995). This class includes the simplest transposable elements called insertion sequences (IS elements), which consist only of a transposase gene and terminal inverted repeat sequences (Mahillon and Chandler, 1998; Siguier *et al.*, 2006). Also, more complex transposable elements that can carry antibiotic resistance genes or catabolic functions between their inverted repeats have DDE transposases. Archetype of complex transposons in Gram-negative bacteria is Tn*3*, which carries resistance to ampicillin, carbenicillin and some other β-lactam antibiotics (Bennett, 1992). Also, Tn*7* family transposons (Hickman *et al.*, 2000), transposable bacteriophages such as Mu (Namgoong and Harshey, 1998) as well as eukaryotic elements (Hua-Van and Capy, 2008) and even retroviruses (Chiu and Davies, 2004) encode for DDE group transposases. Another characteristic of this kind of transposition reaction is target site duplications which arise when transposase-made staggered cuts into the target DNA are repaired after the insertion of an element.

Mobile elements play important role in the evolution of bacterial genomes facilitating generation of genetic variability and horizontal gene transfer (Schneider and Lenski, 2004). The latter is responsible for the quick spread of antibiotic resistance genes between different bacteria (Deng *et al.*, 2009; Roberts and Mullany, 2009). On the other hand, transposons can generate deleterious mutations by insertion within genes, hamper normal gene regulation upon insertion upstream of a gene or generate extensive DNA rearrangements such as inversions or deletions of large DNA segments. Transposition potential to damage the host genome is the reason to maintain these recombinant events at a low level. Therefore, transposition events are usually infrequent and this is mostly achieved by keeping the amount of active transposase in the cell at a low level (Kleckner, 1990). Transposase expression can be repressed by transcrip-

tional or translational control. Additionally, there are examples of post-translational inhibition of the enzyme.

Promoters of most transposases are weak and it is usual that these promoters overlap with terminal inverted repeat sequence which allows auto-repression upon transposase binding to the end (Mahillon and Chandler, 1998). This is demonstrated *in vivo* for *E. coli* resident IS*30* transposase promoter (Dalrymple and Arber, 1985). In case of IS*21* transposase promoter activity was too low to be detectable in the test system (Reimmann *et al.*, 1989). Additionally, some transposase promoters contain transcription termination signals to minimise their expression (Beuzon *et al.*, 1999; Datta and Rosner, 1987) and several mobile elements encode accessory transcription repressor proteins. These negative regulators could be truncated versions of transposase or individual proteins. For example, bacterial insertion sequence IS*1* encodes InsA, which is the N-terminal fragment of transposase capable of binding to the ends of the element and acting as a repressor for transposase promoter as well as transposition reaction (Zerbib *et al.*, 1990). On the other hand, transposon Tn*3* encodes resolvase which binding site overlaps with the promoter of transposase (Sherratt, 1989). Resolvase is necessary for transposition intermediate resolution, but upon binding it can also repress the transcription from transposase gene.

Translational control over transposase expression occurs by many mechanisms. Transposase mRNAs tend to have poor ribosome binding sites that bear little resemblance to the consensus (Kleckner, 1990), or form stem-loop like secondary structures that occlude the ribosome-binding-site (Beuzon *et al.*, 1999; Beuzon *et al.*, 2004; Davis *et al.*, 1985; Krebs and Reznikoff, 1986). Many elements encode antisense RNA which is complementary to transposase mRNA and their pairing effectively down-regulates transposase translation, for instance elements IS*10* and IS*30* (Arini *et al.*, 1997; Case *et al.*, 1989; Case *et al.*, 1990; Simons *et al.*, 1983; Simons and Kleckner, 1983). Sometimes correct expression of transposase needs translational frame-shift between the two open reading frames. As such translational error occurs infrequently this is another effective mechanism to reduce the level of active transposase in order to control transposition (Chandler and Fayet, 1993; Escoubas *et al.*, 1991).

After all these obstacles at transcriptional and translational level, there are additional mechanisms that affect the activity of transposase. Transposase proteins are often unstable and therefore able to act only *in cis*, i.e., in close proximity to the site of synthesis (Kleckner, 1990). It has been shown that the strong preference for action *in cis* in case of IS*903* is due to a very unstable transposase with a physical half-life of only about 3 minutes (Derbyshire *et al.*, 1990). The major determinant in this instability of the IS*903* transposase was the La protease. Unstable transposases are demonstrated also for bacteriophage Mu (Pato and Reich, 1982), transposon Tn*5* (Berg, 1989) and insertion sequence IS*10* (Jain and Kleckner, 1993). There are also cases where other proteins than proteases inhibit transposase action. For example, Tn*5* encodes N-terminally truncated version of transposase which can form a complex with native

transposase and then bind to the ends of the element. Binding of these heterodimers hampers the later stages of the transposition reaction due to the defect in catalysis of DNA cleavage (de la Cruz *et al.*, 1993).

Transposition control is sometimes executed at the level of nucleoprotein complex synapsis with target site. Basically, this means that no catalysis of DNA cleavage occurs before the formation of correct transposition complex between the ends of element, transposase and target. This allows decreasing the number of transposition events to only those that can be successfully finished and limits the number of non-productive cleavages that can damage the host. As an example, transposon Tn*7* exhibits such clever strategy which assures that the excision of element does not occur before the specific target site is assembled to a nucleoprotein complex (Bainton *et al.*, 1991; Skelding *et al.*, 2002). Such target selection strategy suggests that although transposition is often considered a random process, there are several mobile elements that actually choose their target of insertion. In general, transposons can prefer specific DNA sequences (Craig, 1997; Seringhaus *et al.*, 2006; Tobes and Pareja, 2006) or some kind of DNA structures (Mancuso *et al.*, 2010; Minakhina *et al.*, 1999; Parks *et al.*, 2009). Preferred target sequences can be longer (Hu *et al.*, 2001; Olasz *et al.*, 1998) or shorter (Bender and Kleckner, 1992; Goryshin *et al.*, 1998; Higgins *et al.*, 2009; Mizuuchi and Mizuuchi, 1993), well-conserved specific sites (Craig, 1991; Murphy *et al.*, 1991) or quite loose consensuses (Hu *et al.*, 2001; Olasz *et al.*, 1998). Structure targeted by a transposon can be just a bent DNA (Hallet *et al.*, 1994) or structures such as DNA double-strand breaks (Peters and Craig, 2000, 2001), supercoiled DNA (Yigit and Reznikoff, 1999), single- or di-nucleotide mismatches (Yanagihara and Mizuuchi, 2002), triplex DNA (Kuduvalli *et al.*, 2001; Mancuso *et al.*, 2010; Rao *et al.*, 2000), *etc*.

Contrary to target selection, elements can also avoid certain targets. Usually transposons exclude insertions close to a copy of itself and this phenomenon is called target immunity. Complex transposons such as Mu (Adzuma and Mizuuchi, 1989), Tn*7* (DeBoy and Craig, 1996) and Tn*3* (Nicolas *et al.*, 2010; Wiater and Grindley, 1990) use this mechanism in their target selection. Target immunity is useful to survival of transposon as it avoids self-destructive transposition events targeted into its copy. Additionally, several elements avoid actively transcribed regions as their target. This could be a mechanism which allows the transposon to discriminate between the active genes and the silent ones, thus to minimise host damage due to inactivation of essential genes (Craig, 1997). Transposition inhibition by ongoing transcription has been demonstrated for elements such as Tn*5*, Tn*7*, Tn*10* and Mu (Casadesus and Roth, 1989; DeBoy and Craig, 2000; Wang and Higgins, 1994).

In addition to intrinsic control over transposition exerted by an element, several host factors are involved in the regulation of transposition. This allows the adjustment of transposon movement to cell physiology; basically, transposition can be regulated to occur in conditions where cells are the least vulnerable. It is very common that the DNA architectural or histone-like proteins IHF (Integration Host Factor), HU (Heat-Unstable Nucleoid Protein), Fis

(Factor for Inversion Stimulation) and H-NS (Histone-Like Nucleoid Structuring Protein) are involved in transposition in different ways (Chandler and Mahillon, 2002; Mahillon and Chandler, 1998). These small proteins are involved in DNA structuring (Betermier *et al.*, 1994; Luijsterburg *et al.*, 2006) and therefore their role in transposition is also in facilitating the correct assembly of the nucleoprotein complex and in its stability (Swingle *et al.*, 2004; Wiater and Grindley, 1988). There are many examples of host protein involvement in transposition, but most of the elements have not been analysed in sufficient detail to truly understand the combined effects of these proteins. IHF and H-NS usually positively assist transposition process. For example, positive effect of the binding of global regulator H-NS to the transposition complexes of Tn*5* and Tn*10* has been demonstrated (Wardle *et al.*, 2005; Wardle *et al.*, 2009; Whitfield *et al.*, 2009). It has been shown that both IHF and HU promote transposition complex formation in case of bacteriophage Mu (Betermier *et al.*, 1995). On the other hand, Fis protein can inhibit transposition reactions (Betermier *et al.*, 1993; Weinreich and Reznikoff, 1992). Another example is gyrase that has been shown to promote the efficient synapsis of the element ends in case of bacteriophage Mu (Pato and Banerjee, 1996; Pato, 2004). Dam methylase is also often involved in different steps of transposition (Roberts *et al.*, 1985; Yin *et al.*, 1988). Mostly, hemimethylated transposon is more active than the fully methylated one and this is true for transposase promoters and also for transposon ends. This mechanism allows coupling of transposition and replication since DNA is hemimethylated only shortly after replication fork passes. Such strategy is host friendly since the same proteins that are involved in chromosome replication are needed to fulfil the transposition created gaps. Also, homologous recombination can use the replication duplicated chromosome to repair the transposition induced gaps. Timing of transposition according to replication has also an advantage for the spread of transposable element among bacterial population as newly synthesised DNA will be partitioned to progeny cells.

Pseudomonas putida transposon *Tn4652*

P. putida strain PaW1 contains TOL plasmid pWW0 carrying a toluene degradation transposon Tn*4651* (Meulien and Broda, 1982). New *P. putida* strain PaW85 emerged when TOL plasmid pWW0 was lost from the cells and Tn*4652*, a deletion derivative of Tn*4651*, inserted to the chromosome (Tsuda and Iino, 1987). Therefore, *P. putida* strain PaW85, which is isogenic to fully sequenced KT2440 carries 17-kb-long transposon named Tn*4652* in its chromosome. Mobile element Tn*4652* was classified among Tn*3* family because of its replicative transposition mechanism which creates 5-basepair duplications into the target DNA (Tsuda and Iino, 1987). Tn*4652* has 46-bp-long terminal inverted repeats and encodes four proteins involved in its transposition: TnpA transposase (Hõrak and Kivisaar, 1998; Tsuda and Iino, 1987), transposition

repressor TnpC (Hõrak and Kivisaar, 1999) and TnpS and TnpT proteins, which are important in co-integrate resolution through site-specific recombination (Genka *et al.*, 2002; Tsuda *et al.*, 1989). Different factors that have an influence on Tn*4652* transposition activity are summarised in Figure 3.

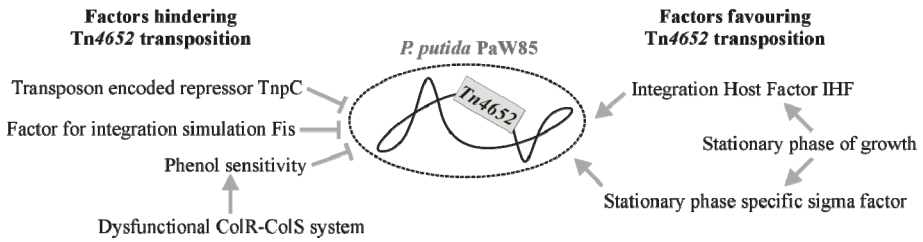

Figure 3. Factors that affect positively or negatively the transpositional activity of mobile element Tn*4652* in *P.putida*.

Transposition of Tn*4652* is heavily down-regulated by TnpC repressor-mediated unknown posttranslational mechanism (Hõrak and Kivisaar, 1999). However, transposition of Tn*4652* is significantly elevated when bacteria reach to the stationary phase of growth (Kasak *et al.*, 1997) and this intriguingly occurs due to several host encoded proteins. First, stationary phase specific sigma factor σ^S is involved in the regulation of Tn*4652* since transposition of Tn*4652* was substantially repressed in cells lacking functional σ^S (Ilves *et al.*, 2001). Additionally, host factors IHF and Fis have controversial roles in regulation of Tn*4652* translocation. Nucleoid protein IHF plays a dual role in activation of Tn*4652* transposition by activating transcription of the transposase gene and facilitating the binding of transposase to the inverted repeats of Tn*4652* (Hõrak and Kivisaar, 1998; Ilves *et al.*, 2004). Fis, on the other hand, is interfering with the binding of IHF and therefore also prohibits the binding of transposase to the end of the element (Teras *et al.*, 2009). It is known that the concentration of IHF and Fis depends on the growth phase of the cells. The amount of IHF is elevated (Valls *et al.*, 2002) and controversially the expression of Fis is down-regulated in stationary phase bacteria (Yuste *et al.*, 2006). Thus, both these factors favour stationary phase specific induction of Tn*4652* movement. Stationary phase cells suffer in hunger and stress over many other things, hence the transposition of Tn*4652* can be also considered as a stress induced process. Additionally, transposition of Tn*4652* was significantly reduced in bacteria with dysfunctional ColR-ColS two-component system (Hõrak *et al.*, 2004). Although, the role of ColR-ColS signal pathway in the regulation of Tn*4652* transposition remained vague and was only seen under phenol starvation conditions, the results suggested that this signal system could affect the target site selection of Tn*4652* (Hõrak *et al.*, 2004). Hence, the target site selection of *P. putida* transposon Tn*4652* was further studied and is discussed in the chapter of Results and Discussion of this thesis.

2. AIMS OF THE STUDY

Our research group is studying the adaptation of environmental bacterium *P. putida* in different stress conditions. My thesis concentrated to the role of ColR-ColS two-component signal pathway that was first identified as a system facilitating root colonisation ability of *P. fluorescens* (Dekkers *et al.*, 1998). ColR-ColS signal pathway is conserved in *Pseudomonas* species, which indicates to its importance. However, processes associated with ColR-ColS pathway in different species vary considerably. For example, ColR-ColS system has been implicated in the virulence of *P. aeruginosa* and heavy metal resistance of *P. putida* (Garvis *et al.*, 2009; Hu and Zhao, 2007). Our group has demonstrated the involvement of ColR-ColS system in transposition of Tn*4652* and the viability of cells growing on glucose solid medium (Hõrak *et al.*, 2004; Putrinš *et al.*, 2008). Yet, the exact target of ColR-ColS pathway has remained obscure. Hence, my study was subjected to elucidating the role of ColR-ColS signal transduction pathway in *P. putida*. The aims of my work can be summarised subsequently:

1. To enlighten the associations between ColR-ColS signal system and transposition of Tn*4652*.
2. To study the target site selection preferences of Tn*4652*.
3. To reveal the target genes of response regulator ColR and to analyse their involvement in phenotypes appearing in case of defective ColR-ColS signalling.

3. RESULTS AND DISCUSSION

Involvement of ColR-ColS signal system in transposition of Tn*4652* (Ref. I and Ref. II)

The first part of my thesis is dedicated to the target site selection of *Pseudomonas putida* mobile element Tn*4652*. Interest in this field arose when my colleagues showed that ColR-ColS two-component signal transduction system is involved in the regulation of Tn*4652* transposition in phenol starvation conditions (Hõrak *et al.*, 2004). Besides seriously reduced transposition frequency, also the insertion site pattern of Tn*4652* had changed somewhat when the transposition events in ColR-deficient cells were compared to those in wild-type *P. putida* (Hõrak *et al.*, 2004). Thus, ColR-ColS system was hypothesised to be involved in the target site selection of Tn*4652*. Transposition of Tn*4652* has been measured in an assay, where *P. putida* cells starving on phenol can acquire phenol-consuming capacity due to the transpositional activation of phenol degradation operon *pheBA* (Ilves *et al.*, 2001). Colonies able to grow on phenol (Phe^+) arise when the movement of chromosomal element Tn*4652* to the target plasmid creates a promoter activating *pheBA* operon. However, this assay allows the detection of very small portion of Tn*4652* target sites, namely only those that create phenol degradation operon activating fusion promoter between the end of transposon and the target DNA (Nurk *et al.*, 1993). Transposition assay that selects only for some particular target sites is biased and actually is not suitable for the target site preference analysis. Hence, to test the effect of ColR-ColS signal system on target site selection of Tn*4652*, I designed a new transposition assay, the mating-out assay, which enables to detect random transposon insertions.

Transposition of Tn*4652* in mating-out assay

Importantly, transposition of Tn*4652* has been examined under phenol starvation conditions because this transposon does not carry any selection markers, which would easily allow tracking its movement. To overcome this limitation, the native Tn*4652* was marked with kanamycin resistance gene (Km^r) and its transposition was studied in a mating-out assay (Ref. I). Mating-out assay reveals the random transposition events of Tn*4652* into the target plasmids as the included kanamycin resistance gene makes the plasmids with inserted Tn*4652* detectable. Mating-out assay demonstrated that Tn*4652* insertions were distributed quite evenly over the target plasmids and no regions were apparently preferred (Ref. I, Fig. 2). Moreover, we did not notice any significant hot-spots for Tn*4652* transposition. However, three target sites were used twice if 96 independent clones were analysed indicating that transposon Tn*4652* could have some target site preferences. Importantly, transposition targets of Tn*4652* did

not coincide with those employed in the phenol starvation assay. These data demonstrated that the target site preferences of Tn*4652* are different in two assays, which is most probably caused by the serious constraints of phenol starvation assay selecting only sites suitable for promoter formation. Here, it is important to remind that the transposition of Tn*4652* was about tenfold reduced in phenol starvation assay if ColR-ColS system was inactivated (Hõrak *et al.*, 2004). Thus, we also compared the transposition frequencies of Tn*4652* in mating-out assay in wild-type *P. putida* and in strain with non-functional ColR-ColS signalling. However, no effect of ColR-ColS pathway on transposition frequency of Tn*4652* was observed in the mating-out assay (unpublished results of Ilves). Hence, the results of mating-out assay do not support the idea that the movement of Tn*4652* could be hindered in the cells with non-functional ColR-ColS signal system. Rather our data is suggesting that the participation of ColR-ColS system in transposition of Tn*4652* is circumstantial e.g. seen only in case of phenol starvation assay. Thus, the effect of ColR-ColS signal system on the transposition of Tn*4652* in the phenol selection assay was taken under study.

Lowered phenol tolerance is behind the decrease in transposition activity of Tn*4652* in ColR-ColS signalling mutant

As already mentioned above, the transposition of mobile element Tn*4652* is hindered in *P. putida* cells in case of hampered ColR-ColS signalling (Hõrak *et al.*, 2004). However, the decrease in transposition frequency of Tn*4652* was seen only in an assay where *P. putida* cells are starved on phenol and not in the mating-out assay. This led us to hypothesise that phenol could be the factor influencing transposition of Tn*4652* in cells with interrupted ColR-ColS signalling. Phenol is a potentially toxic compound that accumulates to bacterial membranes, hence disturbing membrane functioning (Sikkema *et al.*, 1994). Regularly, the transposition of Tn*4652* has been assayed on plates containing 2.5 mM phenol (Ilves *et al.*, 2001). We argued that if phenol toxicity was behind the decreased transposition then smaller phenol concentrations would hinder the transposition of Tn*4652* less. Therefore, the accumulation of Phe^+ transposition mutants was examined on plates with different phenol concentrations to measure the effect of phenol toxicity on transposition (Ref. II, Fig. 6). If wild-type *P. putida* was under the observation then the phenol-growing colonies emerged more or less equally onto the plates with different concentrations of phenol (Ref. II, Fig. 6). At the same time, regularly used 2.5 mM phenol and higher phenol concentrations repressed the accumulation of Phe^+ colonies in cells missing functional ColR (Ref. II, Fig. 6B). However, the emergence of transposition mutants gradually started to increase in ColR-deficient cells when the amount of phenol was lowered to 1 mM or less (Ref. II, Fig. 6B). These results indicate that *P. putida* cells are more susceptible to phenol if the ColR-ColS signal pathway is not functioning. The lowered phenol tolerance of *P. putida* with interrupted ColR-ColS system was additionally confirmed by

minimal inhibitory concentration assay on glucose and citrate plates (Ref. II, Fig. 5). It is known that phenol can cause membrane stress and probably it is the more severe membrane stress in case of ColR absence that somehow leads to the decline of transposition. However, the phenol susceptibility of strain lacking ColR-ColS signalling is not the only factor to be blamed in lowered transposition. Namely, my colleagues have identified phenol tolerant derivatives of the strain with interrupted ColR and noticed that restored phenol tolerance does not necessarily go hand in hand with restored transposition of Tn*4652* (Putrinš *et al.*, 2010b). Taken together, the results of our work suggest that most probably ColR-ColS signal transduction system regulates the transposition of Tn*4652* indirectly, whereas, the exact checkpoint of this system remains to be elucidated. Several lines of evidence indicate that the maintenance of membrane homeostasis is the target of ColR-ColS system (de Weert *et al.*, 2006; Putrinš *et al.*, 2008; Putrinš *et al.*, 2010b). Interestingly, the effect of ColR-ColS signalling was observed on mutational processes occurring in plasmid and not if they were assayed in the chromosome (unpublished results of Ilves and Hõrak). Thus, this signal pathway cannot be directly associated to all mutational processes; rather only the mutations occurring in plasmid could be targeted. Possibly, bacteria lacking ColR have a membrane defect that is highlighted in the presence of phenol and the concurring stress can lead to the alterations in plasmid replication, which is additional load for the bacterium. According to the literature data, replication and partitioning of some plasmids requires binding to the membrane (Firshein and Kim, 1997). Given that the cell membrane could be necessary component of plasmid replication this might explain the suppression of transposition and other mutational processes occurring in such plasmid. However, the copy number of transposition target plasmid was more or less the same in wild-type *P. putida* and cells lacking ColR and this result does not support the hypothesis presented above. Recently, ColR-ColS signalling system has been implicated in the regulation of cell division (Putrinš *et al.*, 2010b). This gives the green light to the hypothesis that colony forming ability of ColR-defect bacteria could be hindered on phenol plates. In this case, the accumulation of Phe^+ colonies would be hampered in cells lacking ColR, but the transposition reaction itself and other mutational processes could be occurring efficiently. Taken together, the results of my work suggest that most probably ColR-ColS signal transduction system regulates the transposition of Tn*4652* indirectly, whereas the checkpoint of this system remains to be elucidated. To address the latter mystery, great part of my thesis concentrates onto the identification of target genes of response regulator ColR.

Target site selection of Tn*4652*

As mentioned above, ColR-ColS pathway did not influence the frequency of transposition of Tn*4652* in mating out assay. Nevertheless, my interest in target site selection had been arisen and several insertion sites of Tn*4652* were already identified leading to a decision to inspect the target preferences and character of transposon Tn*4652* more closely.

My first goal was to shed some light on the orientation bias phenomenon of Tn*4652* transposition. Namely, transposition of Tn*4652* was seen to exhibit significant orientation preference in phenol starvation assay as the fusion promoter was created between the right end of Tn*4652* and the target in more than 90% of insertions (Kasak *et al.*, 1997; Nurk *et al.*, 1993). One could suggest that the right end is better suited for promoter, but it has been shown that the fusion promoters originated from the left end of transposon Tn*4652* are as strong as those generated on the basis of right end (Teras *et al.*, 2000). Orientation of transposon can be influenced by different factors such as the structure of target DNA or the symmetry of transposition complex. There are also examples of transposons that orientate themselves according to the direction of chromosome replication or conjugation (Bernales *et al.*, 1999; Peters and Craig, 2000, 2001). I raised the question whether the orientation preference of Tn*4652* is coupled to the direction of plasmid replication. Transposition target region of Tn*4652* was inversed in respect to the origin of replication in the plasmid to study the correlation of transposition orientation bias and the direction of replication. Transposition of Tn*4652* into original plasmid and into its derivative with inverted *pheBA* region was investigated in parallel in the phenol starvation assay and the study revealed that both plasmids are targeted with equal frequency by Tn*4652* (Ref. I, Fig. 1). In addition, inversion of Tn*4652* target region in plasmid did not change the orientation bias since most of Tn*4652* insertions were still with the transposon right end towards the direction of transcription of *pheBA* genes (Ref. I, Table 2). Yet, the orientation bias of Tn*4652* was switched relative to the direction of replication on *pheBA* region. Consequently, the orientation effect is not caused by the direction of replication on target plasmid; at least not while transposon insertions are observed in phenol starvation assay conditions. It remains unclear which intrinsic properties of Tn*4652* or those of the target region could affect the orientation preference.

Slight orientation bias of Tn*4652* was seen in the mating-out assay as well. About 70% of Tn*4652* insertions were in one orientation in different target plasmids (Ref. I, Fig. 2). Importantly, when observed in the phenol starvation assay the transposon with added Km^r gene oriented in the same manner as the native Tn*4652* (unpublished data). Although these two test systems are not comparable, one can conclude that the orientation bias of Tn*4652* is less strict in mating-out assay. Some processes related to the target plasmids could have the influence on Tn*4652* orientation or it can be the random effect. As already mentioned above, there are some cases where target replication (Bernales *et al.*,

1999; Peters and Craig, 2000, 2001) or the direction of conjugational transfer (Hu and Derbyshire, 1998) affects the orientation of transposon. However, we do not have enough data to decide whether that is true for Tn*4652*.

While establishing the mating-out assay I identified almost hundred independent target sequences of Tn*4652* and comparison of these sequences enabled to create the target consensus for Tn*4652* with a good probability (Ref. I, Fig. 3). This mobile element uses replicative transposition mechanism yielding in 5-bp target duplications (Tsuda and Iino, 1987). These direct repeats of Tn*4652* target DNA contained only A and T nucleotides allowing to sum consensus T(A/T)(T/A)(T/A)(A/T). DNA strands are more easily separated in this type of sequence and that could be beneficial for the transposition event, which involves the breaking and joining reactions of DNA. Contrary to the direct repeats of Tn*4652* insertion sites composed only of A and T nucleotides, the bases flanking the direct repeats are always G or C (Ref. I, Fig. 3). This contrast could facilitate DNA bending in a way, which favours transposase binding and consequent DNA cutting (Hallet *et al.*, 1994). Moreover, the conserved positions in the target sequence of Tn*4652* extend beyond the direct repeat and appear in a palindromic manner (Ref. I, Fig. 3). This is not unusual as several transposases and other DNA binding proteins recognise palindrome sequences (Bender and Kleckner, 1992). The preference for a repeat sequence, whether direct or palindrome, is usually explained with the binding of a dimeric protein wherein each monomer binds one of the repeats. Transposition complex can also be viewed as a dimer consisting of two transposon ends bound by transposase monomers. Actually, the consensus sequence of Tn*4652* identified in this paper (Ref. I, Fig. 3) is very similar to that of its relative transposon Tn*3* (Davies and Hutchison, 1995). Hence, the target selection criteria of Tn*3* family transposons seem to be well conserved and I would suggest that the target site selection is an important character of Tn*4652* transposition despite the huge amount of suitable targets.

Cell membrane is the main target of regulation by ColR-ColS pathway (Ref. II and III)

First identified targets of ColR are membrane porin gene, operon of exo-polysaccharide alginate and an operon putatively involved in membrane functioning

The following part of my thesis is devoted to the identification of target genes of ColR-ColS signal pathway. First, *P. putida* promoter library screening was performed to find the target genes of response regulator ColR (Ref. II, Fig. 1). Library screening was performed on phenol plates because the effect of ColR-ColS system was previously seen in this condition on transposition of Tn*4652*. Thus, we expected that ColR-ColS system is active in these conditions and that

the use of phenol as the carbon source of growth would ease the library search for target genes. After eliminating the false positive results, only the promoters in front of PP0268 and PP1288 were affected by the presence of ColR according to promoter activity measurements (Ref. II, Fig. 2). PP0268 is annotated as outer membrane porin-encoding *oprQ* and PP1288, also called *algD*, as the first gene in 12-gene operon encoding for surface polysaccharide alginate synthesis and degradation functions (www.pseudomonas.com). Both promoters were about 1.7-fold up-regulated in the cells missing a functional ColR if compared to the parent strain. Although, the effect of ColR absence is quite small, the *in vitro* results with purified ColR protein suggest that ColR is directly involved in regulation of these promoters. Gel mobility shift assay demonstrated that purified ColR protein binds to the DNA of PP0268 and PP1288 promoters (Ref. II, Fig. 3). Hence, the small effect of ColR seen *in vivo* could be due to an assay conditions, which do not trigger the full activation of ColR-ColS signal system or the promoters studied.

It is important to point out that both first identified target genes of ColR encode for cell membrane properties. OprQ is an outer membrane porin, which could facilitate the transport of different substances across the outer membrane or participate in the attachment (Hancock and Brinkman, 2002) and ColR-regulated *alg* operon is responsible for the production and degradation of alginate. Alginate and other cell surface polysaccharides are high molecular weight carbohydrate polymers that make up a substantial component of the extracellular polymers surrounding most microbial cells. It has been shown that alginate protects the cell, helps bacteria to colonise surfaces and form biofilm in case of *P. aeruginosa* (Ryder *et al.*, 2007). Thus, if the amount of poly-saccharide alginate is different around the cells lacking functional ColR-ColS pathway, this could very well diminish their root-colonising ability that was shown for *P. fluorescens* strain with impaired ColR-ColS signalling (Dekkers *et al.*, 1998). Actually, it has been shown that *P. syringae* bacteria defective in alginate synthesis are comprised in their ability to colonise tomato and bean leaves (Yu *et al.*, 1999). Interestingly, *algD* promoter was hundred fold up-regulated by plant exudates in maize rhizosphere, whereas, without the induction *algD* promoter was one of the most silent ones (Ramos-Gonzalez *et al.*, 2005). This could indicate that alginate synthesis is initiated in the rhizosphere, establishing important parallel with biofilm lifestyle of *P. aeruginosa* that also depends on alginate (Boyd and Chakrabarty, 1995). Interestingly, we showed that the activity of *algD* promoter was extremely low in both wild-type and ColR-deficient bacteria, but 60-fold induction was registered if the cells lacking functional ColR were facing sub-inhibitory concentration of phenol (Ref. II, Fig. 2). Phenol is an aromatic compound and its structure could resemble some plant derived molecule (Peters and Verma, 1990). Hence, the induction of *algD* promoter in the presence of phenol in ColR-defect bacteria could mimic the effect of plant exudates observed by Ramos-Gonzalez *et al*. (2005). However, there are also some discrepancies

between the two studies as we do not see the induction of *algD* promoter by phenol in wild-type cells.

Concurrently with our publication reporting the first ColR-regulated genes in *P. putida*, de Weert *et al.* (2006) identified the first target gene of ColR in *P. fluorescens*. They showed that ColR-ColS system is regulating an operon downstream of *colRS* genes. This region of a genome is conserved among *Pseudomonas* bacteria and the same operon named PP0903-PP0905 is present in the genome of *P. putida*. Inspired by this conservation in gene arrangement, we measured the activity of PP0903 promoter and registered that it is severely repressed in bacteria lacking functional ColR and significantly activated in cells when ColR is over-expressed (Ref. III, Fig. 1). We also demonstrated the binding of purified ColR to the DNA of this promoter *in vitro* confirming that the promoter of PP0903 is directly ColR-activated. This operon consists of three genes: PP0903 encoding putative methyltransferase, PP0904 encoding lipopolysaccharide kinase InaA and PP0905 encoding hypothetical protein (www.pseudomonas.com). Most likely, proteins encoded by this operon participate in the functioning of membrane since the product of PP0905 is predicted to locate into the cytoplasmic membrane and the product of PP0904 is probably modifying outer membrane lipoglycans. Modifications of outer membrane lipopolysaccharides change cell surface properties and therefore could very well affect the attachment and colonisation of a bacterium. Indeed, the colonisation defect has been shown for ColR-ColS signalling mutant *P. fluorescens* (Dekkers *et al.*, 1998). Actually, the same authors hypothesised that the down-regulation of the ortholog operon of PP0903-PP0905 in *P. fluorescens* deficient in ColR-ColS system is leading to the under-phosphorylated lipo-polysaccharides, which in turn could affect the conformation of membrane porins and impair the cells in competition to root exudates (de Weert *et al.*, 2006). Interestingly, treatment of *P. aeruginosa* with peptidoglycan synthesis inhibiting antibiotic ceftazidime was shown to induce the transcription of a gene encoding lipopolysaccharide kinase InaA (Blazquez *et al.*, 2006). This result suggests that modifications made to the outer membrane lipopolysaccharides by InaA kinase could yield in less permeable membrane, which is necessary for bacterial adaptation and protection against ceftazidime.

Detection of new ColR-regulated genes

Our promoter library screen revealed surprisingly few target genes of ColR-ColS pathway. The two identified ColR-regulated promoters were quite slightly affected by ColR (Ref. II, Fig. 2) and this could indicate that several other target genes of ColR remained under the detection limit of our assay. We used phenol as a carbon source believing that ColR-ColS system is active under this condition. However, we cannot exclude the possibility that the phenol induced membrane stress was actually hampering the detection of ColR-dependent promoters, especially those involved in membrane functioning. Notably, the

genes controlled by the three identified ColR-dependent promoters, *oprQ*, *algD* and PP0903, were disrupted and their involvement in the phenotypes detected in ColR-ColS signalling deficient bacteria was studied by my colleagues. Interruption of these genes did not affect the studied phenotypes of either wild-type or ColR-deficient bacteria (unpublished results of Teesalu and Hõrak). Thus, there were enough reasons to suspect that other genes could be controlled by ColR-ColS signal pathway. Hence, we decided to perform computational search for ColR target genes as a new approach.

Identification of ColR binding consensus

We decided to scan the genome of *P. putida* for putative ColR binding sites. In order to do that, we needed to map the ColR binding sequences in the promoters of previously identified ColR-regulated genes *oprQ*, *algD* and PP0903. ColR binding site was detectable in the promoter region of *oprQ* and PP0903 (Ref. III, Fig. 2). On closer inspection, these two binding sequences of ColR were very similar, having 10 identical positions within 13-bp consensus (Ref. III, Fig. 2B). There is a long T-track in the middle of the site of ColR binding, which correlates to other OmpR family response regulators that tend to have A/T-tracks between direct repeat sequences (de Been *et al.*, 2008; Lejona *et al.*, 2003; Yoshida *et al.*, 2006). The strands of DNA are more loosely bonded in the A/T-rich area and this sequence could take a structure necessary for protein binding. Interestingly, the direct repeat sequence is not very obvious in the ColR binding sites, although there are highly conserved AC pairs separated by nine nucleotides in the ColR recognition sites in promoters of *oprQ* and PP0903. These nucleotides could account for the two direct repeats of ColR binding site if its binding mechanism is similar to that of OmpR. More precisely, OmpR was shown to need GNNACA nucleotides directly repeated after ten nucleotides to make a contact with DNA (Yoshida *et al.*, 2006). Similarly, computational analysis of OmpR family response regulator target motifs in Gram positive *Bacillus* bacteria yielded in the direct repeat TTAAGA separated by four to five nucleotides making the actual distance between the repeats ten to eleven nucleotides (de Been *et al.*, 2008). Nine to ten nucleotides is the length of DNA helix turn, suggesting that both monomers of ColR and other abovementioned OmpR family regulators bind to the same side of DNA. Additionally, it has been demonstrated that differently from some of its homologues OmpR protein can bind DNA in the absence of phosphorylation (Head *et al.*, 1998). The same can be seen in case of ColR binding to different promoters, whereas the phosphorylation of ColR protein increases its affinity to target DNA sequences (Ref. II, Fig. 3; Ref. III, Fig. 2, Fig. 4 and Fig. 5).

There are also indications of ColR multimerisation upon binding as the gel shift experiments demonstrate protein-DNA complexes with different mobility. Here, it is important to point out that these experiments compare the movement of DNA in the presence of different concentrations of protein, whereas the

slowly moving complexes are considered to contain more protein molecules. However, these results are not easy to interpret since the complexes can dissolve during gel mobility shift assay and give misleading patterns (Ref. II, Fig. 3; Ref. III, Fig. 5). The effector modules of OmpR family response regulators are structurally very similar and use the common phosphorylation event, but in sharp contrast, the strategies used to regulate protein activity are different (Birck *et al.*, 2003). Thus, it is not possible to predict the DNA binding mechanism of response regulator ColR based on the literature data, although the significance of dimerisation at some point upon binding is well-established for members of the OmpR subfamily (Fiedler and Weiss, 1995). For example, in case of redox regulator ArcA it has been shown *in vitro* that the un-phosphorylated protein exists as monomer and also forms homodimers, whereas upon phosphorylation large oligomers assemble of dimers, which is a pre-requisite for binding to DNA (Jeon *et al.*, 2001; Toro-Roman *et al.*, 2005). However, the relevance of these oligomers in ArcA binding to DNA *in vivo* could depend on the intracellular conditions, localisation and the presence of DNA and other protein components of transcription complexes. So far, we have no clear evidence whether ColR is binding to DNA at first as a monomer or already as a dimer and whether further oligomerisation of ColR is taking place during its binding to DNA. However, the binding consensus of ColR demonstrates more than two rudimentary repeats (Ref. III, Fig. 6), which could indicate to the binding of multiple molecules, at least in case of some promoters. Despite my efforts, the ColR binding site in the promoter of alginate synthesis operon remained undetectable. Importantly, gel mobility shift assay demonstrated superb binding of ColR to the DNA of *algD* promoter (Ref. II, Fig. 3), which makes the undetectable binding site even more incomprehensible. Actually, there are several sites resembling the ColR binding consensus in the promoter of *algD*, but they are far more dissimilar than those of *oprQ* and PP0903. Thus, it is possible that ColR protein is binding with lower affinity or the dynamics are just somewhat different upon binding to the promoter of *algD* making the interaction impossible to detect with our current footprinting technique. It has been demonstrated that the cellular concentrations of OmpR are 1–3 μM (Cai and Inouye, 2002) and we used the concentration of ColR up to 10 μM in the footprinting experiments (Ref. III, Fig. 2). Hence, I would suggest that the failure to identify the binding site in the promoter of *algD* was not due to the insufficiency of ColR protein, although one can always argue about the functionality of a purified protein *in vitro*. The biggest difference in gel mobility shift and footprint assay is the DNA cutting by DNase in the latter case. Thus, it is possible that the DNA breaking in case of footprint reactions could destabilise the ColR and DNA complex thus far that it yields in the sequestered ColR binding site in the promoter of *algD*.

Novel target genes of regulator ColR

In order to detect new ColR-regulated genes the binding sequences of ColR in the promoters of *oprQ* and PP0903 were aligned (Ref. III, Fig. 2B) and similar sequences were searched from the genome of *P. putida* with the aid of web-based programs (Ref. III). Predicted ColR binding sites were first experimentally validated by promoter activity assay (Ref. III, Fig. 3) and following, confirmed with *in vitro* ColR binding experiments (Ref. III, Fig. 4 and Fig. 5). Computational genome screening for potential ColR binding sequences was made step by step, meaning that several predictions were made and the number of input sequences grew consistently with new verified ColR binding sites. Altogether, five new unique ColR binding sequences were identified in the promoters of PP0035/0036, PP0737, PP0900, PP2560/2561 and PP3766 (Ref. III, Fig. 4). Response regulator ColR activated the expression of these promoters with the exception of PP0737 gene, which activity was down-regulated by ColR (Ref. III, Fig. 3). Thus, transcription factor ColR can act as an activator or repressor for its target genes. Promoter of PP1636 was activated by regulator ColR and demonstrated ColR binding in gel mobility shift assay (Ref. III, Fig. 3 and Fig. 5), but the exact binding site of ColR escaped our analysis similarly to previously assayed *algD* promoter. These new ColR-dependent genes encode for a GtrA family protein (PP0035), LysR family transcriptional regulator (PP0036), putative lipid A 3-O-deacylase PagL (PP0737), PAP2 family protein (PP0900), type I secretion system ATPase (PP2560), heme peroxidase (PP2561), lactoylglutathione lyase GloA (PP3766) and diacylglycerol kinase DgkA-1 (PP1636) according to *Pseudomonas* Database (www.pseudomonas.com).

Several ColR-regulated genes encode proteins involved in membrane homeostasis indicating that the main role of ColR-ColS signal pathway could be insuring the membrane functioning in different conditions. Outer membrane protein PagL has lipid A 3-O-deacylase activity, which modifies lipopolysaccharides by deacylation of lipid A at 3-O-position (Trent *et al.*, 2001). Lipopolysaccharides modified by PagL aid *Salmonella* to resist cationic antimicrobial peptides and avoid recognition by host innate immune system (Kawasaki *et al.*, 2004; Kawasaki *et al.*, 2007). Although, the role of PagL has not been studied in *P. putida*, it could be behind the cation resistance that was lowered in bacteria with non-functional ColR-ColS system (Hu and Zhao, 2007). PP0035 is the first gene of *gtr* operon (PP0035-PP0033) involved in the synthesis of cell surface polysaccharides according to Pseudomonas Database. PP0035 encodes a GtrA family protein that locates to the cytoplasmic membrane and forms a glucosyltransferase complex together with the other two genes of the operon (Korres *et al.*, 2005). This membrane-bound complex has been demonstrated to participate in the lipopolysaccharide exposition on the outer membrane and thus is also responsible for the serotype conversion in *Shigella flexneri* (Korres *et al.*, 2005). The same complex encoded by *gtrABM* has been suggested to participate in membrane maintenance and heavy metal resistance in *Cupriavidus metallidurans* and *P. putida* (Monchy *et al.*, 2007;

Wu *et al.*, 2010). Heavy metal ions can precipitate to the bacterial surface and alter membrane integrity, which can be repaired by the restoration of the outer membrane lipopolysaccharides (Korres *et al.*, 2005; Wu *et al.*, 2010).

Type 2 phosphatidic acid phosphatase (PAP2) and diacylglycerol kinase DgkA encoded by PP0900 and PP1636, respectively, are enzymes embedded to the cytoplasmic membrane and probably affect the composition of membrane lipids. Namely, these enzymes regulate the abundance of phospholipid synthesis precursor phosphatidic acid and membrane-derived oligosaccharide synthesis by-product diacylglycerol (Zhang and Rock, 2008). PP2560 encodes ABC transporter residing in cytoplasmic membrane and constituting a component of type I secretion apparatus exporting degradative enzymes across both inner and outer membranes to the extracellular medium (www.pseudomonas.com). Whereas divergently encoded extracellular heme peroxidase PP2561 could be the protease exported through this secretion system.

ColR target genes that do not encode functions directly associated to bacterial membrane are the enzyme GloA (PP3766) involved in detoxification of a glycolysis side product and LysR family transcriptional regulator (PP0036). Interestingly, the promoter of *gloA* was up-regulated by plant exudates in maize rhizosphere (Ramos-Gonzalez *et al.*, 2005). Considering that this promoter is activated in strain over-expressing ColR protein (Ref. III, Fig. 3) one could speculate about the association between the plant exudates and ColR excess as these two conditions have uniform effect on *gloA* promoter. Possible hypothesis could be that the plant exudates activate the ColR-ColS signalling pathway and thus yield in the similar output as ColR over-expression. However, the signal initiating ColR-ColS system has not been elucidated.

It is noteworthy that response regulator ColR-dependent phosphatase PP0900 locates just upstream of *colRS* genes and is divergently transcribed from PP0901 encoding ColR (www.pseudomonas.com). The detected ColR-binding site affecting the expression of PP0900 promoter is actually located between the divergent PP0900 and *colR* genes. Thus, we also analysed the activity of this promoter in the direction of *colR* (PP0901), but no ColR-responsiveness was seen under any conditions examined (Ref. III, Fig. 3). Therefore, transcription factor ColR does not auto-regulate the expression of its own operon, which is quite unusual for this type of response regulators (Bijlsma and Groisman, 2003; Deng *et al.*, 2010).

Predicted regulon of ColR-ColS system includes over 40 genes

ColR-regulated genes described in the previous chapter were disrupted with the exception of PP0035 and these mutants were analysed for the participation in the phenotypes of ColR-ColS signalling defect bacteria such as phenol susceptibility, lysis on glucose and reduced transposition of Tn*4652* (unpublished results of Teesalu and Hõrak). These data suggested that ColR-regulated genes identified thus far were not involved in the studied phenotypes of bacteria with defective ColR. However, it is possible that these phenotypes are not caused by only one deregulated gene; rather, they could be the outcome of the complex interplay between the dysfunction of several genes. On the other hand, the range of ColR-ColS signal pathway could be wider than anticipated meaning that the whole regulon of this system was not yet identified. To predict the new genes of ColR regulon seven already identified ColR binding sequences were aligned and the binding consensus was composed (Ref. III, Fig. 6). Expectedly, the new ColR binding consensus demonstrated the same features discussed already above on the promoters of *oprQ* and PP903. Fortunately, the first two ColR binding sites were highly similar (Ref. III, Fig. 2) and that allowed us to use strict criteria in the search for similar sites decreasing the occurrence of false-positives. However, the harsh parameters of the search could be discriminating for the sites less similar to the first ones but still able to bind ColR *in vivo*. Therefore, the predicted regulon of ColR is considered minimal and should be interpreted with caution.

Prediction of the minimal regulon of ColR-ColS pathway in *P. putida* with the aid of ColR consensus motif of seven sequences revealed that apparently more than 40 genes are affected by this system (Ref. III, Additional file 1). Functions of the genes that belong to the regulon of ColR-ColS system are recited in the Table 1 and their involvement in the phenotypes of bacteria lacking functional ColR are discussed in the following chapter about the checkpoint of ColR-ColS pathway. However, briefly stated about two thirds of putative ColR binding sites locate upstream of genes implicated in membrane functioning further confirming our conclusion that the main role of ColR-ColS signalling pathway is to safeguard the bacterial cell wall and membrane.

Interestingly, ColR-regulated promoters PP1692 and PP2322/PP2323 contain multiple sites similar to ColR consensus sequence suggesting that their promoter activity could be finely modulated depending on the differential binding of ColR in response to different conditions. Thus, it is possible that response regulator ColR has a binding mode similar to that of OmpR, which has many different affinity binding sites in the promoters of its target genes *ompF* and *ompC* (Pratt *et al.*, 1996). Multiple binding sites create the dynamics for simultaneous activation of one gene and repression of the other.

Table 1. Target genes of response regulator ColR.

CYTOPLASMIC MEMBRANE PROTEINS:
PP0905[a], **PP2828**[b] and **PP4086**
PP2579 sulfatase
PP1487 tellurium resistance protein
Transporters: PP2560 ABC transporter
PP1059 amino acid transporter
PP1259 amino acid ABC transporter permease
PP2628 ABC transporter ATP binding protein
PP3953 cation transport protein

OUTER MEMBRANE PROTEINS:
Porins: PP0268 and **PP2322**
PP0677 lipoprotein
PP0267 ferric siderophore receptor
PP3536 type IV secretion system chaperone
PP4057 type V secretion system transporter

PERIPLASMIC BINDING PROTEINS:
PP1486 and PP4305

LIPOPOLYSACCHARIDE AND LIPID COMPONENT:
PP0035-PP0033 glucosyltransferase complex
PP0737 lipid A 3-O deacylase
PP0900 phosphatidic acid phosphatase
PP0904 lipopolysaccharide kinase
PP1636 diacylglycerol kinase
PP1915 acyl carrier protein
PP4538 acyl carrier protein phosphodiesterase
PP4286 polysaccharide deacetylase

CELL SURFACE:
PP1288 alginate biosynthesis protein
PP2561 secreted hemolysin
PP0592 extracellular dehydrogenase
PP2986 oxidoreductase, sugar epimerase

CELL WALL SYNTHESIS:
PP1058 penicillin binding protein
PP2630 cellulose biosynthesis protein

CHEMOTAXIS AND MOVEMENT:
PP1819 chemotaxis transducer
PP4378 flagellum component

MEMBRANE FUNCTIONS

TRANSCRIPTION:
PP0036 transcriptional regulator
PP0676 transcription elongation factor
PP2946 peptidyl tRNA hydrolase
PP4539 transcriptional regulator
PP5108 RNA polymerase sigma factor H

METABOLISM:
PP3766 lactoylglutathione lyase
PP0751 malate oxidoreductase
PP2339 aconitate hydratase
PP4285 transthyretin family protein

DNA METABOLISM:
PP2139 DNA topoisomerase I
PP3260 DNA ligase

OTHER PROCESSES

[a] experimentally verified ColR-regulated genes are in black
[b] genes predicted to be ColR-regulated are in grey

Potential checkpoint of ColR-ColS system in membrane homeostasis

ColR-ColS signal transduction pathway has been demonstrated to participate in different processes such as the colonisation of *P. fluorescens* and the virulence of *P. aeruginosa* (Dekkers *et al.*, 1998; Garvis *et al.*, 2009). Our research group has been studying the role of ColR-ColS system in *P. putida* and discovered its involvement in the phenol tolerance and concurrently in the transposition of Tn*4652* (Ref. II; Hõrak *et al.*, 2004). Furthermore, proper ColR-ColS signalling is needed for the viability of *P. putida* under certain conditions, particularly when bacteria are growing on solid glucose medium (Putrinš *et al.*, 2008; Putrinš *et al.*, 2010b). This thesis has revealed several target genes of response regulator ColR in *P. putida* (Table 1) giving us an opportunity to explain how ColR-ColS signal pathway could affect all these different processes.

The main target of ColR-ColS system appears to be bacterial membrane according to the regulon of ColR (Table 1). The cell envelope of Gram negative bacteria is unique by its composition as it consists of two membranes. The outer membrane is composed of lipopolysaccharides (LPS) in addition to phospholipids that are the typical component of membranes. High negative charge of lipopolysaccharides is stabilised by the membrane bound cations, which are

mostly Mg^{2+} ions (Hancock, 1997). This structure protects the cells effectively as the membrane LPS layer is a good barrier to hydrophilic molecules and the LPS bound cations prevent the attachment of hydrophobic molecules. However, cells need exchange of hydrophilic molecules and therefore the lipid component of the membrane hides several porin and efflux proteins (Hancock and Brinkman, 2002). Two membranes of Gram negative bacteria are separated by the periplasmic space that is also the residence of peptidoglycan cell wall. Additionally, cells can be surrounded with the polysaccharide capsule which is protecting them against desiccation. Thus, there are different compartments in the bacterial membrane that could be affected by the ColR-ColS system. The overview of bacterial cell envelope was recently given by Silhavy *et al.* (2010).

Current thesis has demonstrated that the cells with non-functional ColR-ColS system are less tolerant to phenol than the wild-type (Ref. II, Fig. 5). Additionally, we have seen the indirect indications of phenol susceptibility of bacteria with interrupted ColR-ColS system as several promoters are affected by phenol in cells with defective ColR, but not in wild-type background (Ref. II, Fig. 2; Ref. III, Fig. 1 and Fig. 3). Also, the transposition of Tn*4652* was inhibited by phenol only in cells lacking functional ColR (Ref. II, Fig. 6). Phenol is a molecule that accumulates into the membrane and disrupts its function (Sikkema *et al.*, 1995). It has been shown that bacteria encountering phenol activate specific response, which includes up-regulation of energy metabolism, fatty acid and cell envelope synthesis, and several proteins of stress response and efflux, whereas, membrane porins and processes like cell division and mobility are down-regulated (Roma-Rodrigues *et al.*, 2010; Santos *et al.*, 2004). Integrity of the membrane is vital for the cell and thus bacteria possess mechanisms allowing them to sense the condition of cell envelope and to respond appropriately to maintain the membrane homeostasis. Well-characterised example of bacterial envelope stress response is alternative sigma factor σ^E mediated regulation triggered by the accumulation of misfolded outer membrane proteins (OMP) (Rowley *et al.*, 2006; Ruiz and Silhavy, 2005). Activation of the σ^E pathway causes a rapid down-regulation of major OMP mRNAs and thus prevents the further build-up of unassembled proteins in the membrane. Another example of envelope stress response system in *E. coli* is CpxR-CpxA two-component pathway, which regulates the expression of porins (Batchelor *et al.*, 2005). One could assume similar role for ColR-ColS system in *Pseudomonas* as the main targets affected by response regulator ColR are membrane proteins, lipid component of the membrane, cell wall, surface polysaccharides, mobility and transcription (Table 1). Additionally, ColR-mediated regulation was enhanced in the presence of membrane disturbing agent phenol confirming the hypothesis that ColR-ColS signal pathway could sense envelope stress and mediate the response to repair the membrane.

Aborted ColR-ColS signalling was demonstrated to lead to cell lysis of *P. putida* growing on glucose (Putrinš *et al.*, 2008; Putrinš *et al.*, 2010a). Different signals can initiate the cell lysis, which culminates in cell envelope disintegration releasing the cellular proteins to the environment (Asakura and

Kobayashi, 2009). Thus, cell envelope defects lead to lysis if not repaired in time and we have plenty of reasons to suspect that the bacteria with defective ColR suffer due to the disturbed membrane. Interestingly, only a subpopulation of glucose-growing ColR-mutant lyses and the recent results suggest that particularly only the subpopulation encountering glucose deprivation autolyses in case of defective ColR-ColS signal transduction (Putrinš *et al.*, 2010a). Importantly, cells that are starving, i.e. those that do not grow, do not escalate to lysis (Putrinš *et al.*, 2010b). The degree of autolyses inversely depended on the glucose concentration, which lead to the hypothesis that lysis was occurring due to the initiation of hunger response in cells sensing glucose limitation. Accumulation of glucose import porin OprB1 in glucose limitation conditions was demonstrated to elevate the autolysis of bacteria with defective ColR-ColS signalling (Putrinš *et al.*, 2010a). OprB1 protein is locating in the outer membrane and thus this compartment of bacterial cell could be altered in case of dysfunctional ColR-ColS system. However, it is not easy to distinguish whether the lipid component of membrane could be somehow defective and thus unable to accommodate proteins properly or the protein component of ColR-deficient cells itself is acting against additional OprB1 molecules. Both options are plausible as the regulon of ColR-ColS system contains several genes for membrane proteins and genes involved in the homeostasis of lipids (Table 1). Remarkably high portion of ColR regulon genes are implicated in the regulation of membrane lipid component. Namely, in addition to identified ColR target genes discussed above, the predicted regulon of ColR contains PP1915 putatively coding for acyl carrier protein AcpP, which is involved in fatty acid and phospholipid metabolism (www.pseudomonas.com). The product of PP4286 belongs to the family of polysaccharide deacetylases that remove acetyl group from sugar. This set of the genes points to the lipids as a target, which homeostasis is regulated by ColR-ColS system. However, similar patterns of lipopolysaccharides were observed if the membranes of ColR-mutant bacteria and its parent strain were analysed (unpublished results of Putrinš). Additionally, we have interrupted several target genes of ColR (PP0900, PP0904, PP0737, PP1636) involved in the synthesis or modification of lipids (unpublished). Yet, these genes do not seem to participate in the phenotypes appearing in case of defective ColR-ColS pathway. However, many outer membrane proteins are also affected by the dysfunctional ColR-ColS signalling (Table 1). The predicted regulon of ColR includes outer membrane porins PP0268 and PP2322, lipoprotein with unknown function (PP0677), secretion system type V autotransporter (PP4057), ATP-dependent type VI secretion system chaperone (PP3536) and ferric siderophore receptor (PP0267). Another possibility is that the cooperative effect of lipids and proteins results in the sensitisation of the outer membrane of ColR-mutant meaning that the ratio of proteins to lipids is important for accurate membrane functioning.

Additionally, the regulon of response regulator ColR is containing genes that encode functions of other membrane compartments. For example, peptidoglycan cell wall synthesis could be affected by ColR-ColS system as such a role

has been suggested to ColR target gene PP1058. This is a probable penicillin-binding protein whose transglycosylase domain catalyses the polymerisation of murein chains of peptidoglycan layer (Ishidate *et al.*, 1998). Cell wall determines the shape of bacterium and gives the necessary strength to resist the osmotic pressure of the cytoplasm (Silhavy *et al.*, 2010). Interestingly, the expression of confirmed ColR target gene PP904 was induced in the presence of cell wall mucopeptide synthesis-inhibiting antibiotic ceftazidime in *P. aeruginosa* (Blazquez *et al.*, 2006). In this light, one could suggest that ColR-ColS system is reacting to the defects in cell wall synthesis or is counteracting them. Damaged peptidoglycan of bacteria lacking ColR could lead to cell lysis due to the turgor pressure of the cytoplasm. Nevertheless, it is rather unlikely that ColR-ColS signalling could significantly affect the synthesis of cell wall in *P. puitda* since the bacteria defective in ColR-ColS system demonstrated no vulnerability in the presence of antibiotics hampering the synthesis of peptidoglycan (unpublished). There are also genes facilitating the synthesis of alginate and cellulose in the regulon of ColR. These two polysaccharides are known to form the capsule conferring the protection and facilitating cell adhesion during symbiotic or infectious interactions in various bacterial species (Ross *et al.*, 1987; Ross *et al.*, 1991). Altered capsule surrounding the cells could also explain the decreased colonisation properties of *P. fluorescens* and virulence of *P. aeruginosa* observed in the absence of ColR-ColS signal transduction (Dekkers *et al.*, 1998; Garvis *et al.*, 2009). Capsule also protects the bacterial cell from the harassing environment including desiccation, detergents, *etc*., which could explain the fact that *P. putida* lacking functional ColR turned up to be sensitive to several divalent heavy metal ions (Hu and Zhao, 2007).

In conclusion, ColR-ColS signal transduction pathway is mainly regulating membrane functions, whereas the data about the regulon of ColR is indicating to membrane protein and lipid component as well as to cell wall peptidoglycan and exopolysaccharides alginate and cellulose as the compartments affected by regulator ColR. Thus, it is highly probable the sensor ColS could be registering the welfare of cell envelope and its partner ColR regulates functions that can counteract the membrane disturbances inflicted by noxious stressors.

CONCLUSIONS

First part of my thesis studied the involvement of ColR-ColS signal system in the transposition of Tn*3*-family mobile element Tn*4652*. The results of this part can be summarised with the following conclusions:

- ColR-ColS signal system affects the transposition of Tn*4652* indirectly.
  - Transposition of Tn*4652* was tenfold down-regulated in cells with interrupted ColR-ColS signalling (Hõrak *et al.*, 2004). The transposition of Tn*4652* has been measured in bacteria starving on phenol and the accumulation of phenol utilising transposition mutants (Phe^+) was assayed. Current study demonstrated that the transposition of Tn*4652* depended on the concentration of phenol in the medium employed for the selection of mutants; the higher the phenol concentration was, the more the accumulation of transposition mutants was repressed in ColR-mutant strain. These data suggest that bacteria defective in ColR-ColS signalling are more susceptible to phenol than the wild-type and that the toxic effect of phenol could be behind the decrease in transposition.
  - Mating-out assay was created to detect the transposition of Tn*4652* without the necessity to employ phenol selection. Transposition of Tn*4652* was not affected by the presence of functional ColR-ColS system in this assay.
- In addition, the target site preferences of Tn*4652* were compared in two assays. The targets of Tn*4652* in mating-out assay allowed drawing a consensus similar to that of Tn*3*. These data indicate that the target site selection criteria are conserved among Tn*3* family members.

Second part of my thesis is dedicated to the role of ColR-ColS signal transduction system in *P. putida* and to the identification of target genes of response regulator ColR. This study can be taken together with the following conclusions:

- The main target of ColR-ColS signal pathway is the bacterial membrane.
  - Most of the identified or predicted target genes of ColR encode for membrane functions, whereas different membrane compartments appeared to be affected. The regulon of ColR contains genes encoding for outer and inner membrane proteins and genes, whose products are involved in lipid and fatty acid metabolism, and peptidoglycan or exopolysaccharide synthesis. Thus, both the lipid and protein component of the membrane are affected by ColR-ColS system, as well as the peptidoglycan cell wall and polysaccharide capsule.
- Binding sequences of response regulator ColR are similar to the direct repeats of other OmpR family proteins suggesting that the binding of ColR to the DNA needs protein dimerisation or even further multimerisation.

REFERENCES

Adzuma, K., and Mizuuchi, K. (1989) Interaction of proteins located at a distance along DNA: mechanism of target immunity in the Mu DNA strand-transfer reaction. *Cell* **57**: 41–47.

Albanesi, D., Martin, M., Trajtenberg, F., Mansilla, M.C., Haouz, A., Alzari, P.M., de Mendoza, D., and Buschiazzo, A. (2009) Structural plasticity and catalysis regulation of a thermosensor histidine kinase. *Proc Natl Acad Sci U S A* **106**: 16185–16190.

Anand, G.S., Goudreau, P.N., Lewis, J.K., and Stoc, A.M. (2000) Evidence for phosphorylation-dependent conformational changes in methylesterase CheB. *Protein Sci* **9**: 898–906.

Anzai, Y., Kim, H., Park, J.Y., Wakabayashi, H., and Oyaizu, H. (2000) Phylogenetic affiliation of the pseudomonads based on 16S rRNA sequence. *Int J Syst Evol Microbiol* **50 Pt 4**: 1563–1589.

Appleby, J.L., and Bourret, R.B. (1998) Proposed signal transduction role for conserved CheY residue Thr87, a member of the response regulator active-site quintet. *J Bacteriol* **180**: 3563–3569.

Arini, A., Keller, M.P., and Arber, W. (1997) An antisense RNA in IS*30* regulates the translational expression of the transposase. *Biol Chem* **378**: 1421–1431.

Asakura, Y., and Kobayashi, I. (2009) From damaged genome to cell surface: transcriptome changes during bacterial cell death triggered by loss of a restriction-modification gene complex. *Nucleic Acids Res* **37**: 3021–3031.

Atkinson, S., and Williams, P. (2009) Quorum sensing and social networking in the microbial world. *J R Soc Interface* **6**: 959–978.

Bader, M.W., Sanowar, S., Daley, M.E., Schneider, A.R., Cho, U., Xu, W., Klevit, R.E., Le Moual, H., and Miller, S.I. (2005) Recognition of antimicrobial peptides by a bacterial sensor kinase. *Cell* **122**: 461–472.

Bainton, R., Gamas, P., and Craig, N.L. (1991) Tn*7* transposition in vitro proceeds through an excised transposon intermediate generated by staggered breaks in DNA. *Cell* **65**: 805–816.

Baker, M.D., Wolanin, P.M., and Stock, J.B. (2006) Signal transduction in bacterial chemotaxis. *Bioessays* **28**: 9–22.

Barrow, K., and Kwon, D.H. (2009) Alterations in two-component regulatory systems of phoPQ and pmrAB are associated with polymyxin B resistance in clinical isolates of Pseudomonas aeruginosa. *Antimicrob Agents Chemother* **53**: 5150–5154.

Batchelor, E., Walthers, D., Kenney, L.J., and Goulian, M. (2005) The Escherichia coli CpxA-CpxR envelope stress response system regulates expression of the porins ompF and ompC. *J Bacteriol* **187**: 5723–5731.

Bechthold, A. (2005) Exploiting Pseudomonas putida for drug development. *Chem Biol* **12**: 261.

Bender, J., and Kleckner, N. (1992) Tn*10* insertion specificity is strongly dependent upon sequences immediately adjacent to the target-site consensus sequence. *Proc Natl Acad Sci U S A* **89**: 7996–8000.

Bennett, P.M. (1992) Transposable elements. *Encyclopedia of Microbiology* **4**: 311–325.

Berg, D.E. (1989) Transposon Tn*5*. In *Mobile DNA*. Berg, D.E. and Howe, M.M. (eds). Washington, D. C.: American Society for Microbiology, pp. 185–210.

Bergstrom, L.C., Qin, L., Harlocker, S.L., Egger, L.A., and Inouye, M. (1998) Hierarchical and co-operative binding of OmpR to a fusion construct containing the

ompC and ompF upstream regulatory sequences of Escherichia coli. *Genes Cells* **3**: 777–788.

Berman, M.L., and Jackson, D.E. (1984) Selection of lac gene fusions in vivo: ompR-lacZ fusions that define a functional domain of the ompR gene product. *J Bacteriol* **159**: 750–756.

Bernales, I., Mendiola, M.V., and de la Cruz, F. (1999) Intramolecular transposition of insertion sequence IS91 results in second-site simple insertions. *Mol Microbiol* **33**: 223–234.

Betermier, M., Poquet, I., Alazard, R., and Chandler, M. (1993) Involvement of *Escherichia coli* FIS protein in maintenance of bacteriophage mu lysogeny by the repressor: control of early transcription and inhibition of transposition. *J Bacteriol* **175**: 3798–3811.

Betermier, M., Galas, D.J., and Chandler, M. (1994) Interaction of Fis protein with DNA: bending and specificity of binding. *Biochimie* **76**: 958–967.

Betermier, M., Rousseau, P., Alazard, R., and Chandler, M. (1995) Mutual stabilisation of bacteriophage Mu repressor and histone-like proteins in a nucleoprotein structure. *J Mol Biol* **249**: 332–341.

Beuzon, C.R., Marques, S., and Casadesus, J. (1999) Repression of IS*200* transposase synthesis by RNA secondary structures. *Nucleic Acids Res* **27**: 3690–3695.

Beuzon, C.R., Chessa, D., and Casadesus, J. (2004) IS*200*: an old and still bacterial transposon. *Int Microbiol* **7**: 3–12.

Bijlsma, J.J., and Groisman, E.A. (2003) Making informed decisions: regulatory interactions between two-component systems. *Trends Microbiol* **11**: 359–366.

Birck, C., Chen, Y., Hulett, F.M., and Samama, J.P. (2003) The crystal structure of the phosphorylation domain in PhoP reveals a functional tandem association mediated by an asymmetric interface. *J Bacteriol* **185**: 254–261.

Bjarnsholt, T., Jensen, P.O., Jakobsen, T.H., Phipps, R., Nielsen, A.K., Rybtke, M.T., Tolker-Nielsen, T., Givskov, M., Hoiby, N., and Ciofu, O. (2010) Quorum sensing and virulence of Pseudomonas aeruginosa during lung infection of cystic fibrosis patients. *PLoS One* **5**: e10115.

Blazquez, J., Gomez-Gomez, J.M., Oliver, A., Juan, C., Kapur, V., and Martin, S. (2006) PBP3 inhibition elicits adaptive responses in *Pseudomonas aeruginosa*. *Mol Microbiol* **62**: 84–99.

Bouche, S., Klauck, E., Fischer, D., Lucassen, M., Jung, K., and Hengge-Aronis, R. (1998) Regulation of RssB-dependent proteolysis in Escherichia coli: a role for acetyl phosphate in a response regulator-controlled process. *Mol Microbiol* **27**: 787–795.

Bourret, R.B. (2010) Receiver domain structure and function in response regulator proteins. *Curr Opin Microbiol* **13**: 142–149.

Boyd, A., and Chakrabarty, A.M. (1995) *Pseudomonas aeruginosa* biofilms: role of the alginate exopolysaccharide. *J Ind Microbiol.* **15**: 162–168.

Brencic, A., McFarland, K.A., McManus, H.R., Castang, S., Mogno, I., Dove, S.L., and Lory, S. (2009) The GacS/GacA signal transduction system of Pseudomonas aeruginosa acts exclusively through its control over the transcription of the RsmY and RsmZ regulatory small RNAs. *Mol Microbiol* **73**: 434–445.

Brzostek, K., Brzostkowska, M., Bukowska, I., Karwicka, E., and Raczkowska, A. (2007) OmpR negatively regulates expression of invasin in Yersinia enterocolitica. *Microbiology* **153**: 2416–2425.

Buell, C.R., Joardar, V., Lindeberg, M., Selengut, J., Paulsen, I.T., Gwinn, M.L., Dodson, R.J., Deboy, R.T., Durkin, A.S., Kolonay, J.F., Madupu, R., Daugherty, S.,

Brinkac, L., Beanan, M.J., Haft, D.H., Nelson, W.C., Davidsen, T., Zafar, N., Zhou, L., Liu, J., Yuan, Q., Khouri, H., Fedorova, N., Tran, B., Russell, D., Berry, K., Utterback, T., Van Aken, S.E., Feldblyum, T.V., D'Ascenzo, M., Deng, W.L., Ramos, A.R., Alfano, J.R., Cartinhour, S., Chatterjee, A.K., Delaney, T.P., Lazarowitz, S.G., Martin, G.B., Schneider, D.J., Tang, X., Bender, C.L., White, O., Fraser, C.M., and Collmer, A. (2003) The complete genome sequence of the Arabidopsis and tomato pathogen Pseudomonas syringae pv. tomato DC3000. *Proc Natl Acad Sci U S A* **100**: 10181–10186.

Busch, A., Lacal, J., Martos, A., Ramos, J.L., and Krell, T. (2007) Bacterial sensor kinase TodS interacts with agonistic and antagonistic signals. *Proc Natl Acad Sci U S A* **104**: 13774–13779.

Cai, S.J., and Inouye, M. (2002) EnvZ-OmpR interaction and osmoregulation in Escherichia coli. *J Biol Chem* **277**: 24155–24161.

Canovas, D., Cases, I., and de Lorenzo, V. (2003) Heavy metal tolerance and metal homeostasis in Pseudomonas putida as revealed by complete genome analysis. *Environ Microbiol* **5**: 1242–1256.

Casadesus, J., and Roth, J.R. (1989) Transcriptional occlusion of transposon targets. *Mol Gen Genet* **216**: 204–209.

Case, C.C., Roels, S.M., Jensen, P.D., Lee, J., Kleckner, N., and Simons, R.W. (1989) The unusual stability of the IS*10* anti-sense RNA is critical for its function and is determined by the structure of its stem-domain. *Embo J* **8**: 4297–4305.

Case, C.C., Simons, E.L., and Simons, R.W. (1990) The IS*10* transposase mRNA is destabilized during antisense RNA control. *Embo J* **9**: 1259–1266.

Chandler, M., and Fayet, O. (1993) Translational frameshifting in the control of transposition in bacteria. *Mol Microbiol* **7**: 497–503.

Chandler, M., and Mahillon, J. (2002) Insertion sequences revisited. In *Mobile DNA II*. Craig, N.L., Craigie, R., Gellert, M. and M., L.A. (eds). Washington, D. C.: American Society for Microbiology, pp. 305–366.

Chang, C., and Stewart, R.C. (1998) The two-component system. Regulation of diverse signaling pathways in prokaryotes and eukaryotes. *Plant Physiol* **117**: 723–731.

Cheng, H.Y., Chen, Y.F., and Peng, H.L. (2010) Molecular characterization of the PhoPQ-PmrD-PmrAB mediated pathway regulating polymyxin B resistance in Klebsiella pneumoniae CG43. *J Biomed Sci* **17**: 60.

Cheung, J., and Hendrickson, W.A. (2010) Sensor domains of two-component regulatory systems. *Curr Opin Microbiol* **13**: 116–123.

Cheung, J.K., Keyburn, A.L., Carter, G.P., Lanckriet, A.L., Van Immerseel, F., Moore, R.J., and Rood, J.I. (2010) The VirSR two-component signal transduction system regulates NetB toxin production in Clostridium perfringens. *Infect Immun* **78**: 3064–3072.

Chiu, T.K., and Davies, D.R. (2004) Structure and function of HIV-1 integrase. *Curr Top Med Chem* **4**: 965–977.

Choi, E., Groisman, E.A., and Shin, D. (2009) Activated by different signals, the PhoP/PhoQ two-component system differentially regulates metal uptake. *J Bacteriol* **191**: 7174–7181.

Comeau, D.E., Ikenaka, K., Tsung, K.L., and Inouye, M. (1985) Primary characterization of the protein products of the Escherichia coli ompB locus: structure and regulation of synthesis of the OmpR and EnvZ proteins. *J Bacteriol* **164**: 578–584.

Costerton, J.W., Stewart, P.S., and Greenberg, E.P. (1999) Bacterial biofilms: a common cause of persistent infections. *Science* **284**: 1318–1322.

Cowan, S.W., Schirmer, T., Rummel, G., Steiert, M., Ghosh, R., Pauptit, R.A., Jansonius, J.N., and Rosenbusch, J.P. (1992) Crystal structures explain functional properties of two E. coli porins. *Nature* **358**: 727–733.

Craig, N.L. (1991) Tn*7*: a target site-specific transposon. *Mol Microbiol* **5**: 2569–2573.

Craig, N.L. (1997) Target site selection in transposition. *Annu Rev Biochem* **66**: 437–474.

Dalrymple, B., and Arber, W. (1985) Promotion of RNA transcription on the insertion element IS30 of E. coli K12. *Embo J* **4**: 2687–2693.

Datta, A.R., and Rosner, J.L. (1987) Reduced transposition in rho mutants of Escherichia coli K-12. *J Bacteriol* **169**: 888–890.

Davies, C.J., and Hutchison, C.A., 3rd (1995) Insertion site specificity of the transposon Tn*3*. *Nucleic Acids Res* **23**: 507–514.

Davis, M.A., Simons, R.W., and Kleckner, N. (1985) Tn*10* protects itself at two levels from fortuitous activation by external promoters. *Cell* **43**: 379–387.

de Been, M., Bart, M.J., Abee, T., Siezen, R.J., and Francke, C. (2008) The identification of response regulator-specific binding sites reveals new roles of two-component systems in *Bacillus cereus* and closely related low-GC Gram-positives. *Environ Microbiol* **10**: 2796–2809.

de la Cruz, N.B., Weinreich, M.D., Wiegand, T.W., Krebs, M.P., and Reznikoff, W.S. (1993) Characterization of the Tn*5* transposase and inhibitor proteins: a model for the inhibition of transposition. *J Bacteriol* **175**: 6932–6938.

De Weert, S., Dekkers, L.C., Kuiper, I., Bloemberg, G.V., and Lugtenberg, B.J. (2004) Generation of Enhanced Competitive Root-Tip-Colonizing *Pseudomonas* Bacteria through Accelerated Evolution. *J Bacteriol* **186**: 3153–3159.

de Weert, S., Dekkers, L.C., Bitter, W., Tuinman, S., Wijfjes, A.H., van Boxtel, R., and Lugtenberg, B.J. (2006) The two-component *colR/S* system of *Pseudomonas fluorescens* WCS365 plays a role in rhizosphere competence through maintaining the structure and function of the outer membrane. *FEMS Microbiol Ecol* **58**: 205–213.

DeBoy, R.T., and Craig, N.L. (1996) Tn*7* transposition as a probe of cis interactions between widely separated (190 kilobases apart) DNA sites in the *Escherichia coli* chromosome. *J Bacteriol* **178**: 6184–6191.

DeBoy, R.T., and Craig, N.L. (2000) Target site selection by Tn*7*: *att*Tn*7* transcription and target activity. *J Bacteriol* **182**: 3310–3313.

Dekkers, L.C., Bloemendaal, C.J., de Weger, L.A., Wijffelman, C.A., Spaink, H.P., and Lugtenberg, B.J. (1998) A two-component system plays an important role in the root-colonizing ability of *Pseudomonas fluorescens* strain WCS365. *Mol Plant Microbe Interact* **11**: 45–56.

Deng, X., Lan, L., Xiao, Y., Kennelly, M., Zhou, J.M., and Tang, X. (2010) Pseudomonas syringae two-component response regulator RhpR regulates promoters carrying an inverted repeat element. *Mol Plant Microbe Interact* **23**: 927–939.

Deng, Y., Zeng, Z., Liu, J., and Chen, Z. (2009) Insertion sequence common region element: a novel gene-capturing system in bacteria. *Wei Sheng Wu Xue Bao* **49**: 987–993.

Derbyshire, K.M., Kramer, M., and Grindley, N.D. (1990) Role of instability in the cis action of the insertion sequence IS*903* transposase. *Proc Natl Acad Sci U S A* **87**: 4048–4052.

Dorel, C., Lejeune, P., and Rodrigue, A. (2006) The Cpx system of *Escherichia coli*, a strategic signaling pathway for confronting adverse conditions and for settling biofilm communities? *Res Microbiol* **157**: 306–314.

Dorman, C.J., Chatfield, S., Higgins, C.F., Hayward, C., and Dougan, G. (1989) Characterization of porin and ompR mutants of a virulent strain of Salmonella typhimurium: ompR mutants are attenuated in vivo. *Infect Immun* **57**: 2136–2140.

Drevinek, P., Baldwin, A., Lindenburg, L., Joshi, L.T., Marchbank, A., Vosahlikova, S., Dowson, C.G., and Mahenthiralingam, E. (2010) Oxidative stress of Burkholderia cenocepacia induces insertion sequence-mediated genomic rearrangements that interfere with macrorestriction-based genotyping. *J Clin Microbiol* **48**: 34–40.

Duan, K., Dammel, C., Stein, J., Rabin, H., and Surette, M.G. (2003) Modulation of *Pseudomonas aeruginosa* gene expression by host microflora through interspecies communication. *Mol Microbiol* **50**: 1477–1491.

Duan, K., Sibley, C.D., Davidson, C.J., and Surette, M.G. (2009) Chemical interactions between organisms in microbial communities. *Contrib Microbiol* **16**: 1–17.

Dubuis, C., and Haas, D. (2007) Cross-species GacA-controlled induction of antibiosis in pseudomonads. *Appl Environ Microbiol* **73**: 650–654.

Dutta, R., Qin, L., and Inouye, M. (1999) Histidine kinases: diversity of domain organization. *Mol Microbiol* **34**: 633–640.

Eguchi, Y., Itou, J., Yamane, M., Demizu, R., Yamato, F., Okada, A., Mori, H., Kato, A., and Utsumi, R. (2007) B1500, a small membrane protein, connects the two-component systems EvgS/EvgA and PhoQ/PhoP in Escherichia coli. *Proc Natl Acad Sci U S A* **104**: 18712–18717.

Eichenbaum, Z., and Livneh, Z. (1998) UV light induces IS*10* transposition in *Escherichia coli*. *Genetics* **149**: 1173–1181.

Ernst, R.K., Guina, T., and Miller, S.I. (2001) *Salmonella typhimurium* outer membrane remodeling: role in resistance to host innate immunity. *Microbes Infect.* **3**: 1327–1334.

Escoubas, J.M., Prere, M.F., Fayet, O., Salvignol, I., Galas, D., Zerbib, D., and Chandler, M. (1991) Translational control of transposition activity of the bacterial insertion sequence IS*1*. *Embo J* **10**: 705–712.

Espinosa-Urgel, M., Kolter, R., and Ramos, J.L. (2002) Root colonization by Pseudomonas putida: love at first sight. *Microbiology* **148**: 341–343.

Falke, J.J., Bass, R.B., Butler, S.L., Chervitz, S.A., and Danielson, M.A. (1997) The two-component signaling pathway of bacterial chemotaxis: a molecular view of signal transduction by receptors, kinases, and adaptation enzymes. *Annu Rev Cell Dev Biol* **13**: 457–512.

Fernandez, L., Gooderham, W.J., Bains, M., McPhee, J.B., Wiegand, I., and Hancock, R.E. (2010) Adaptive resistance to the "last hope" antibiotics polymyxin B and colistin in Pseudomonas aeruginosa is mediated by the novel two-component regulatory system ParR-ParS. *Antimicrob Agents Chemother* **54**: 3372–3382.

Ferrario, M., Ernsting, B.R., Borst, D.W., Wiese, D.E., 2nd, Blumenthal, R.M., and Matthews, R.G. (1995) The leucine-responsive regulatory protein of Escherichia coli negatively regulates transcription of ompC and micF and positively regulates translation of ompF. *J Bacteriol* **177**: 103–113.

Fiedler, U., and Weiss, V. (1995) A common switch in activation of the response regulators NtrC and PhoB: phosphorylation induces dimerization of the receiver modules. *Embo J* **14**: 3696–3705.

Firshein, W., and Kim, P. (1997) Plasmid replication and partition in *Escherichia coli*: is the cell membrane the key? *Mol Microbiol.* **23**: 1–10.

Fisher, S.L., Kim, S.K., Wanner, B.L., and Walsh, C.T. (1996) Kinetic comparison of the specificity of the vancomycin resistance VanSfor two response regulators, VanR and PhoB. *Biochemistry* **35**: 4732–4740.

Forst, S., Delgado, J., and Inouye, M. (1989) Phosphorylation of OmpR by the osmosensor EnvZ modulates expression of the ompF and ompC genes in *Escherichia coli*. *Proc Natl Acad Sci U S A* **86**: 6052–6056.

Fraser, C.M., Gocayne, J.D., White, O., Adams, M.D., Clayton, R.A., Fleischmann, R.D., Bult, C.J., Kerlavage, A.R., Sutton, G., Kelley, J.M., Fritchman, R.D., Weidman, J.F., Small, K.V., Sandusky, M., Fuhrmann, J., Nguyen, D., Utterback, T.R., Saudek, D.M., Phillips, C.A., Merrick, J.M., Tomb, J.F., Dougherty, B.A., Bott, K.F., Hu, P.C., Lucier, T.S., Peterson, S.N., Smith, H.O., Hutchison, C.A., 3rd, and Venter, J.C. (1995) The minimal gene complement of Mycoplasma genitalium. *Science* **270**: 397–403.

Galperin, M.Y. (2004) Bacterial signal transduction network in a genomic perspective. *Environ Microbiol* **6**: 552–567.

Galperin, M.Y. (2006) Structural classification of bacterial response regulators: diversity of output domains and domain combinations. *J Bacteriol* **188**: 4169–4182.

Galperin, M.Y. (2010) Diversity of structure and function of response regulator output domains. *Curr Opin Microbiol* **13**: 150–159.

Garcia Vescovi, E., Soncini, F.C., and Groisman, E.A. (1994) The role of the PhoP/PhoQ regulon in Salmonella virulence. *Res Microbiol* **145**: 473–480.

Garcia Vescovi, E., Soncini, F.C., and Groisman, E.A. (1996) Mg2+ as an extracellular signal: environmental regulation of *Salmonella virulence*. *Cell* **84**: 165–174.

Garvis, S., Munder, A., Ball, G., de Bentzmann, S., Wiehlmann, L., Ewbank, J.J., Tümmler, B., and Filloux, A. (2009) *Caenorhabditis elegans* semi-automated liquid screen reveals a specialized role for the chemotaxis gene *cheB2* in *Pseudomonas aeruginosa* virulence. *PLoS Pathog* **5**: e1000540.

Genka, H., Nagata, Y., and Tsuda, M. (2002) Site-specific recombination system encoded by toluene catabolic transposon Tn*4651*. *J Bacteriol* **184**: 4757–4766.

Gerken, H., Charlson, E.S., Cicirelli, E.M., Kenney, L.J., and Misra, R. (2009) MzrA: a novel modulator of the EnvZ/OmpR two-component regulon. *Mol Microbiol* **72**: 1408–22.

Gong, W., Hao, B., Mansy, S.S., Gonzalez, G., Gilles-Gonzalez, M.A., and Chan, M.K. (1998) Structure of a biological oxygen sensor: a new mechanism for heme-driven signal transduction. *Proc Natl Acad Sci U S A* **95**: 15177–15182.

Gong, W., Hao, B., and Chan, M.K. (2000) New mechanistic insights from structural studies of the oxygen-sensing domain of Bradyrhizobium japonicum FixL. *Biochemistry* **39**: 3955–3962.

Gooderham, W.J., and Hancock, R.E. (2009) Regulation of virulence and antibiotic resistance by two-component regulatory systems in *Pseudomonas aeruginosa*. *FEMS Microbiol Rev* **33**: 279–294.

Goodman, A.L., Kulasekara, B., Rietsch, A., Boyd, D., Smith, R.S., and Lory, S. (2004) A signaling network reciprocally regulates genes associated with acute infection and chronic persistence in *Pseudomonas aeruginosa*. *Dev Cell* **7**: 745–754.

Goodman, A.L., Merighi, M., Hyodo, M., Ventre, I., Filloux, A., and Lory, S. (2009) Direct interaction between sensor kinase proteins mediates acute and chronic disease phenotypes in a bacterial pathogen. *Genes Dev* **23**: 249–259.

Goryshin, I.Y., Miller, J.A., Kil, Y.V., Lanzov, V.A., and Reznikoff, W.S. (1998) Tn*5*/IS*50* target recognition. *Proc Natl Acad Sci U S A* **95**: 10716–10721.

Gosset, G. (2009) Production of aromatic compounds in bacteria. *Curr Opin Biotechnol* **20**: 651–658.

Groisman, E.A. (2001) The pleiotropic two-component regulatory system PhoP-PhoQ. *J Bacteriol* **183**: 1835–1842.

Gross, R., Arico, B., and Rappuoli, R. (1989) Families of bacterial signal-transducing proteins. *Mol Microbiol* **3**: 1661–1667.
Guillier, M., and Gottesman, S. (2006) Remodelling of the *Escherichia coli* outer membrane by two small regulatory RNAs. *Mol Microbiol.* **59**: 231–247.
Haas, D., Keel, C., and Reimmann, C. (2002) Signal transduction in plant-beneficial rhizobacteria with biocontrol properties. *Antonie Van Leeuwenhoek* **81**: 385–395.
Hallet, B., Rezsohazy, R., Mahillon, J., and Delcour, J. (1994) IS*231*A insertion specificity: consensus sequence and DNA bending at the target site. *Mol Microbiol* **14**: 131–139.
Hancock, R.E. (1997) The bacterial outer membrane as a drug barrier. *Trends Microbiol* **5**: 37–42.
Hancock, R.E. (1998) Resistance mechanisms in Pseudomonas aeruginosa and other nonfermentative gram-negative bacteria. *Clin Infect Dis* **27 Suppl 1**: S93–99.
Hancock, R.E., and Brinkman, F.S. (2002) Function of *pseudomonas* porins in uptake and efflux. *Annu Rev Microbiol* **56**: 17–38.
Haren, L., Ton-Hoang, B., and Chandler, M. (1999) Integrating DNA: transposases and retroviral integrases. *Annu Rev Microbiol* **53**: 245–281.
Harlocker, S.L., Bergstrom, L., and Inouye, M. (1995) Tandem binding of six OmpR proteins to the *ompF* upstream regulatory sequence of *Escherichia coli*. *J Biol Chem* **270**: 26849–26856.
Head, C.G., Tardy, A., and Kenney, L.J. (1998) Relative binding affinities of OmpR and OmpR-phosphate at the ompF and ompC regulatory sites. *J Mol Biol* **281**: 857–870.
Heeb, S., and Haas, D. (2001) Regulatory roles of the GacS/GacA two-component system in plant-associated and other gram-negative bacteria. *Mol Plant Microbe Interact* **14**: 1351–1363.
Herrera, A., and Cockell, C.S. (2007) Exploring microbial diversity in volcanic environments: a review of methods in DNA extraction. *J Microbiol Methods* **70**: 1–12.
Hickman, A.B., Li, Y., Mathew, S.V., May, E.W., Craig, N.L., and Dyda, F. (2000) Unexpected structural diversity in DNA recombination: the restriction endonuclease connection. *Mol Cell* **5**: 1025–1034.
Hickman, A.B., Chandler, M., and Dyda, F. (2010) Integrating prokaryotes and eukaryotes: DNA transposases in light of structure. *Crit Rev Biochem Mol Biol* **45**: 50–69.
Higgins, B.P., Popkowski, A.C., Caruana, P.R., and Karls, A.C. (2009) Site-specific insertion of IS492 in Pseudoalteromonas atlantica. *J Bacteriol* **191**: 6408–6414.
Hirano, S.S., and Upper, C.D. (2000) Bacteria in the leaf ecosystem with emphasis on Pseudomonas syringae-a pathogen, ice nucleus, and epiphyte. *Microbiol Mol Biol Rev* **64**: 624–653.
Hoch, J.A. (2000) Two-component and phosphorelay signal transduction. *Curr Opin Microbiol* **3**: 165–170.
Hoiby, N., Bjarnsholt, T., Givskov, M., Molin, S., and Ciofu, O. (2010) Antibiotic resistance of bacterial biofilms. *Int J Antimicrob Agents* **35**: 322–332.
Hong, H.J., Hutchings, M.I., and Buttner, M.J. (2008) Vancomycin resistance VanS/VanR two-component systems. *Adv Exp Med Biol* **631**: 200–213.
Hrabak, E.M., and Willis, D.K. (1992) The lemA gene required for pathogenicity of Pseudomonas syringae pv. syringae on bean is a member of a family of two-component regulators. *J Bacteriol* **174**: 3011–3020.
Hu, N., and Zhao, B. (2007) Key genes involved in heavy-metal resistance in *Pseudomonas putida* CD2. *FEMS Microbiol Lett* **267**: 17–22.

Hu, W.Y., and Derbyshire, K.M. (1998) Target choice and orientation preference of the insertion sequence IS*903*. *J Bacteriol* **180**: 3039–3048.
Hu, W.Y., Thompson, W., Lawrence, C.E., and Derbyshire, K.M. (2001) Anatomy of a preferred target site for the bacterial insertion sequence IS*903*. *J Mol Biol* **306**: 403–416.
Hua-Van, A., and Capy, P. (2008) Analysis of the DDE motif in the Mutator superfamily. *J Mol Evol* **67**: 670–681.
Humair, B., Wackwitz, B., and Haas, D. (2010) GacA-controlled activation of promoters for small RNA genes in Pseudomonas fluorescens. *Appl Environ Microbiol* **76**: 1497–1506.
Hwang, I., Thorgeirsson, T., Lee, J., Kustu, S., and Shin, Y.K. (1999) Physical evidence for a phosphorylation-dependent conformational change in the enhancer-binding protein NtrC. *Proc Natl Acad Sci U S A* **96**: 4880–4885.
Hõrak, R., and Kivisaar, M. (1998) Expression of the transposase gene *tnpA* of Tn*4652* is positively affected by integration host factor. *J Bacteriol* **180**: 2822–2829.
Hõrak, R., and Kivisaar, M. (1999) Regulation of the transposase of Tn*4652* by the transposon-encoded protein TnpC. *J Bacteriol* **181**: 6312–6318.
Hõrak, R., Ilves, H., Pruunsild, P., Kuljus, M., and Kivisaar, M. (2004) The ColR-ColS two-component signal transduction system is involved in regulation of Tn*4652* transposition in *Pseudomonas putida* under starvation conditions. *Mol Microbiol* **54**: 795–807.
Igo, M.M., and Silhavy, T.J. (1988) EnvZ, a transmembrane environmental sensor of *Escherichia coli* K-12, is phosphorylated in vitro. *J Bacteriol* **170**: 5971–5973.
Igo, M.M., Ninfa, A.J., Stock, J.B., and Silhavy, T.J. (1989) Phosphorylation and dephosphorylation of a bacterial transcriptional activator by a transmembrane receptor. *Genes Dev* **3**: 1725–1734.
Ilves, H., Hõrak, R., and Kivisaar, M. (2001) Involvement of sigma(S) in starvation-induced transposition of *Pseudomonas putida* transposon Tn*4652*. *J Bacteriol* **183**: 5445–5448.
Ilves, H., Hõrak, R., Teras, R., and Kivisaar, M. (2004) IHF is the limiting host factor in transposition of *Pseudomonas putida* transposon Tn*4652* in stationary phase. *Mol Microbiol* **51**: 1773–1785.
Ishidate, K., Ursinus, A., Holtje, J.V., and Rothfield, L. (1998) Analysis of the length distribution of murein glycan strands in ftsZ and ftsI mutants of E. coli. *FEMS Microbiol Lett* **168**: 71–75.
Jain, C., and Kleckner, N. (1993) Preferential cis action of IS*10* transposase depends upon its mode of synthesis. *Mol Microbiol* **9**: 249–260.
Jenal, U. (2000) Signal transduction mechanisms in Caulobacter crescentus development and cell cycle control. *FEMS Microbiol Rev* **24**: 177–191.
Jenal, U., and Galperin, M.Y. (2009) Single domain response regulators: molecular switches with emerging roles in cell organization and dynamics. *Curr Opin Microbiol* **12**: 152–160.
Jeon, Y., Lee, Y.S., Han, J.S., Kim, J.B., and Hwang, D.S. (2001) Multimerization of phosphorylated and non-phosphorylated ArcA is necessary for the response regulator function of the Arc two-component signal transduction system. *J Biol Chem* **276**: 40873–40879.
Jin, T., and Inouye, M. (1993) Ligand binding to the receptor domain regulates the ratio of kinase to phosphatase activities of the signaling domain of the hybrid Escherichia coli transmembrane receptor, Taz1. *J Mol Biol* **232**: 484–492.

Kagle, J., Porter, A.W., Murdoch, R.W., Rivera-Cancel, G., and Hay, A.G. (2009) Biodegradation of pharmaceutical and personal care products. *Adv Appl Microbiol* **67**: 65–108.

Kasak, L., Hõrak, R., and Kivisaar, M. (1997) Promoter-creating mutations in *Pseudomonas putida*: a model system for the study of mutation in starving bacteria. *Proc Natl Acad Sci U S A* **94**: 3134–3139.

Kato, A., and Groisman, E.A. (2008) The PhoQ/PhoP regulatory network of Salmonella enterica. *Adv Exp Med Biol* **631**: 7–21.

Kawasaki, K., Ernst, R.K., and Miller, S.I. (2004) 3-O-deacylation of lipid A by PagL, a PhoP/PhoQ-regulated deacylase of *Salmonella typhimurium*, modulates signaling through Toll-like receptor 4. *J Biol Chem* **279**: 20044–20048.

Kawasaki, K., China, K., and Nishijima, M. (2007) Release of the lipopolysaccharide deacylase PagL from latency compensates for a lack of lipopolysaccharide amino-arabinose modification-dependent resistance to the antimicrobial peptide polymyxin B in *Salmonella enterica*. *J Bacteriol* **189**: 4911–4919.

Kay, E., Dubuis, C., and Haas, D. (2005) Three small RNAs jointly ensure secondary metabolism and biocontrol in Pseudomonas fluorescens CHA0. *Proc Natl Acad Sci U S A* **102**: 17136–17141.

Kay, E., Humair, B., Denervaud, V., Riedel, K., Spahr, S., Eberl, L., Valverde, C., and Haas, D. (2006) Two GacA-dependent small RNAs modulate the quorum-sensing response in *Pseudomonas aeruginosa*. *J Bacteriol* **188**: 6026–6033.

Kennedy, A.K., Haniford, D.B., and Mizuuchi, K. (2000) Single active site catalysis of the successive phosphoryl transfer steps by DNA transposases: insights from phosphorothioate stereoselectivity. *Cell* **101**: 295–305.

Kenney, L.J. (2002) Structure/function relationships in OmpR and other winged-helix transcription factors. *Curr Opin Microbiol.* **5**: 135–141.

Kertesz, M.A., and Mirleau, P. (2004) The role of soil microbes in plant sulphur nutrition. *J Exp Bot* **55**: 1939–1945.

Khorchid, A., and Ikura, M. (2006) Bacterial histidine kinase as signal sensor and transducer. *Int J Biochem Cell Biol.* **38**: 307–312.

Kivisaar, M. (2003) Stationary phase mutagenesis: mechanisms that accelerate adaptation of microbial populations under environmental stress. *Environ Microbiol* **5**: 814–827.

Kleckner, N. (1990) Regulation of transposition in bacteria. *Annu Rev Cell Biol* **6**: 297–327.

Korres, H., Mavris, M., Morona, R., Manning, P.A., and Verma, N.K. (2005) Topological analysis of GtrA and GtrB proteins encoded by the serotype-converting cassette of Shigella flexneri. *Biochem Biophys Res Commun* **328**: 1252–1260.

Krebs, M.P., and Reznikoff, W.S. (1986) Transcriptional and translational initiation sites of IS*50*. Control of transposase and inhibitor expression. *J Mol Biol* **192**: 781–791.

Kuduvalli, P.N., Rao, J.E., and Craig, N.L. (2001) Target DNA structure plays a critical role in Tn*7* transposition. *Embo J* **20**: 924–932.

Lacal, J., Busch, A., Guazzaroni, M.E., Krell, T., and Ramos, J.L. (2006) The TodS-TodT two-component regulatory system recognizes a wide range of effectors and works with DNA-bending proteins. *Proc Natl Acad Sci U S A* **103**: 8191–8196.

Laguri, C., Phillips-Jones, M.K., and Williamson, M.P. (2003) Solution structure and DNA binding of the effector domain from the global regulator PrrA (RegA) from Rhodobacter sphaeroides: insights into DNA binding specificity. *Nucleic Acids Res* **31**: 6778–6787.

Lapouge, K., Sineva, E., Lindell, M., Starke, K., Baker, C.S., Babitzke, P., and Haas, D. (2007) Mechanism of hcnA mRNA recognition in the Gac/Rsm signal transduction pathway of Pseudomonas fluorescens. *Mol Microbiol* **66**: 341–356.

Lapouge, K., Schubert, M., Allain, F.H., and Haas, D. (2008) Gac/Rsm signal transduction pathway of gamma-proteobacteria: from RNA recognition to regulation of social behaviour. *Mol Microbiol* **67**: 241–253.

Laville, J., Voisard, C., Keel, C., Maurhofer, M., Defago, G., and Haas, D. (1992) Global control in Pseudomonas fluorescens mediating antibiotic synthesis and suppression of black root rot of tobacco. *Proc Natl Acad Sci U S A* **89**: 1562–1566.

Lebeau, A., Reverchon, S., Gaubert, S., Kraepiel, Y., Simond-Cote, E., Nasser, W., and Van Gijsegem, F. (2008) The GacA global regulator is required for the appropriate expression of Erwinia chrysanthemi 3937 pathogenicity genes during plant infection. *Environ Microbiol* **10**: 545–559.

Lee, A.K., Detweiler, C.S., and Falkow, S. (2000) OmpR regulates the two-component system SsrA-ssrB in Salmonella pathogenicity island 2. *J Bacteriol* **182**: 771–781.

Lejona, S., Aguirre, A., Cabeza, M.L., Garcia Vescovi, E., and Soncini, F.C. (2003) Molecular characterization of the Mg2+-responsive PhoP-PhoQ regulon in Salmonella enterica. *J Bacteriol* **185**: 6287–6294.

Lopez, D., and Kolter, R. (2010) Extracellular signals that define distinct and coexisting cell fates in Bacillus subtilis. *FEMS Microbiol Rev* **34**: 134–149.

Luijsterburg, M.S., Noom, M.C., Wuite, G.J., and Dame, R.T. (2006) The architectural role of nucleoid-associated proteins in the organization of bacterial chromatin: a molecular perspective. *J Struct Biol* **156**: 262–272.

Lukat, G.S., McCleary, W.R., Stock, A.M., and Stock, J.B. (1992) Phosphorylation of bacterial response regulator proteins by low molecular weight phospho-donors. *Proc Natl Acad Sci U S A.* **89**: 718–722.

Magnusson, L.U., Farewell, A., and Nystrom, T. (2005) ppGpp: a global regulator in Escherichia coli. *Trends Microbiol* **13**: 236–242.

Mahillon, J., and Chandler, M. (1998) Insertion sequences. *Microbiol Mol Biol Rev* **62**: 725–774.

Mancuso, M., Sammarco, M.C., and Grabczyk, E. (2010) Transposon Tn7 preferentially inserts into GAA*TTC triplet repeats under conditions conducive to Y*R*Y triplex formation. *PLoS One* **5**: e11121.

Martin, M., Albanesi, D., Alzari, P.M., and de Mendoza, D. (2009) Functional in vitro assembly of the integral membrane bacterial thermosensor DesK. *Protein Expr Purif* **66**: 39–45.

Martinez-Hackert, E., and Stock, A.M. (1997) The DNA-binding domain of OmpR: crystal structures of a winged helix transcription factor. *Structure* **5**: 109–124.

Mascher, T., Helmann, J.D., and Unden, G. (2006) Stimulus perception in bacterial signal-transducing histidine kinases. *Microbiol Mol Biol Rev* **70**: 910–938.

Mennecier, S., Servant, P., Coste, G., Bailone, A., and Sommer, S. (2006) Mutagenesis via IS transposition in *Deinococcus radiodurans*. *Mol Microbiol.* **59**: 317–325.

Meulien, P., and Broda, P. (1982) Identification of chromosomally integrated TOL DNA in cured derivatives of *Pseudomonas putida* PAW1. *J Bacteriol* **152**: 911–914.

Miller, S.I., Kukral, A.M., and Mekalanos, J.J. (1989) A two-component regulatory system (phoP phoQ) controls Salmonella typhimurium virulence. *Proc Natl Acad Sci U S A* **86**: 5054–5058.

Minakhina, S., Kholodii, G., Mindlin, S., Yurieva, O., and Nikiforov, V. (1999) Tn*5053* family transposons are *res* site hunters sensing plasmidal *res* sites occupied by cognate resolvases. *Mol Microbiol* **33**: 1059–1068.

Mizuno, T., and Mizushima, S. (1990) Signal transduction and gene regulation through the phosphorylation of two regulatory components: the molecular basis for the osmotic regulation of the porin genes. *Mol Microbiol* **4**: 1077–1082.

Mizuno, T. (1997) Compilation of all genes encoding two-component phosphotransfer signal transducers in the genome of *Escherichia coli*. *DNA Res* **4**: 161–168.

Mizuno, T. (2005) Two-component phosphorelay signal transduction systems in plants: from hormone responses to circadian rhythms. *Biosci Biotechnol Biochem.* **69**: 2263–2276.

Mizuuchi, M., and Mizuuchi, K. (1993) Target site selection in transposition of phage Mu. *Cold Spring Harb Symp Quant Biol* **58**: 515–523.

Monchy, S., Benotmane, M.A., Janssen, P., Vallaeys, T., Taghavi, S., van der Lelie, D., and Mergeay, M. (2007) Plasmids pMOL28 and pMOL30 of Cupriavidus metallidurans are specialized in the maximal viable response to heavy metals. *J Bacteriol* **189**: 7417–7425.

Monsieurs, P., De Keersmaecker, S., Navarre, W.W., Bader, M.W., De Smet, F., McClelland, M., Fang, F.C., De Moor, B., Vanderleyden, J., and Marchal, K. (2005) Comparison of the PhoPQ regulon in Escherichia coli and Salmonella typhimurium. *J Mol Evol* **60**: 462–474.

Moon, K., and Gottesman, S. (2009) A PhoQ/P-regulated small RNA regulates sensitivity of Escherichia coli to antimicrobial peptides. *Mol Microbiol* **74**: 1314–1330.

Murata, T., Tseng, W., Guina, T., Miller, S.I., and Nikaido, H. (2007) PhoPQ-mediated regulation produces a more robust permeability barrier in the outer membrane of *Salmonella enterica* serovar typhimurium. *J Bacteriol* **189**: 7213–7222.

Murphy, E., Reinheimer, E., and Huwyler, L. (1991) Mutational analysis of att554, the target of the site-specific transposon Tn554. *Plasmid* **26**: 20–29.

Naas, T., Blot, M., Fitch, W.M., and Arber, W. (1995) Dynamics of IS-related genetic rearrangements in resting *Escherichia coli* K-12. *Mol Biol Evol* **12**: 198–207.

Nakka, S., Qi, M., and Zhao, Y. (2010) The Erwinia amylovora PhoPQ system is involved in resistance to antimicrobial peptide and suppresses gene expression of two novel type III secretion systems. *Microbiol Res* **165**: 665–673.

Namgoong, S.Y., and Harshey, R.M. (1998) The same two monomers within a MuA tetramer provide the DDE domains for the strand cleavage and strand transfer steps of transposition. *Embo J* **17**: 3775–3785.

Nelson, K.E., Weinel, C., Paulsen, I.T., Dodson, R.J., Hilbert, H., Martins dos Santos, V.A., Fouts, D.E., Gill, S.R., Pop, M., Holmes, M., Brinkac, L., Beanan, M., DeBoy, R.T., Daugherty, S., Kolonay, J., Madupu, R., Nelson, W., White, O., Peterson, J., Khouri, H., Hance, I., Chris Lee, P., Holtzapple, E., Scanlan, D., Tran, K., Moazzez, A., Utterback, T., Rizzo, M., Lee, K., Kosack, D., Moestl, D., Wedler, H., Lauber, J., Stjepandic, D., Hoheisel, J., Straetz, M., Heim, S., Kiewitz, C., Eisen, J.A., Timmis, K.N., Dusterhoft, A., Tummler, B., and Fraser, C.M. (2002) Complete genome sequence and comparative analysis of the metabolically versatile *Pseudomonas putida* KT2440. *Environ Microbiol* **4**: 799–808.

Newcombe, J., Jeynes, J.C., Mendoza, E., Hinds, J., Marsden, G.L., Stabler, R.A., Marti, M., and McFadden, J.J. (2005) Phenotypic and transcriptional characterization of the meningococcal PhoPQ system, a magnesium-sensing two-component regulatory system that controls genes involved in remodeling the meningococcal cell surface. *J Bacteriol* **187**: 4967–4975.

Nichols, C.A., Guezennec, J., and Bowman, J.P. (2005) Bacterial exopolysaccharides from extreme marine environments with special consideration of the southern ocean,

sea ice, and deep-sea hydrothermal vents: a review. *Mar Biotechnol (NY)* **7**: 253–271.
Nicolas, E., Lambin, M., and Hallet, B. (2010) Target immunity of the Tn3-family transposon Tn4430 requires specific interactions between the transposase and the terminal inverted repeats of the transposon. *J Bacteriol* **192**: 4233–4238.
Nikaido, H., Rosenberg, E.Y., and Foulds, J. (1983) Porin channels in Escherichia coli: studies with beta-lactams in intact cells. *J Bacteriol* **153**: 232–240.
Ninfa, A.J., and Magasanik, B. (1986) Covalent modification of the *glnG* product, NRI, by the glnL product, NRII, regulates the transcription of the *glnALG* operon in *Escherichia coli*. *Proc Natl Acad Sci U S A*. **83**: 5909–5913.
Nurk, A., Tamm, A., Hõrak, R., and Kivisaar, M. (1993) In-vivo-generated fusion promoters in *Pseudomonas putida*. *Gene* **127**: 23–29.
O'Sullivan, D.J., and O'Gara, F. (1992) Traits of fluorescent Pseudomonas spp. involved in suppression of plant root pathogens. *Microbiol Rev* **56**: 662–676.
Ogura, M., Tsukahara, K., and Tanaka, T. (2010) Identification of the sequences recognized by the Bacillus subtilis response regulator YclJ. *Arch Microbiol* **192**: 569–580.
Ohtsubo, Y., Genka, H., Komatsu, H., Nagata, Y., and Tsuda, M. (2005) High-Temperature-Induced Transposition of Insertion Elements in *Burkholderia multivorans* ATCC 17616. *Appl Environ Microbiol* **71**: 1822–1828.
Olasz, F., Kiss, J., Konig, P., Buzas, Z., Stalder, R., and Arber, W. (1998) Target specificity of insertion element IS*30*. *Mol Microbiol* **28**: 691–704.
Oshima, T., Aiba, H., Masuda, Y., Kanaya, S., Sugiura, M., Wanner, B.L., Mori, H., and Mizuno, T. (2002) Transcriptome analysis of all two-component regulatory system mutants of *Escherichia coli* K-12. *Mol Microbiol* **46**: 281–291.
Otto, M. (2009) Bacterial sensing of antimicrobial peptides. *Contrib Microbiol* **16**: 136–149.
Overgaard, M., Kallipolitis, B., and Valentin-Hansen, P. (2009) Modulating the bacterial surface with small RNAs: a new twist on PhoP/Q-mediated lipopolysaccharide modification. *Mol Microbiol* **74**: 1289–1294.
Palleroni, N.J. (1984) *Genus I. Pseudomonas Migula 1894*. Baltimore: Williams and Wilkins Co.
Palleroni, N.J. (2010) The pseudomonas story. *Environ Microbiol* **12**: 1377–1383.
Park, D., and Forst, S. (2006) Co-regulation of motility, exoenzyme and antibiotic production by the EnvZ-OmpR-FlhDC-FliA pathway in Xenorhabdus nematophila. *Mol Microbiol* **61**: 1397–1412.
Parks, A.R., Li, Z., Shi, Q., Owens, R.M., Jin, M.M., and Peters, J.E. (2009) Transposition into replicating DNA occurs through interaction with the processivity factor. *Cell* **138**: 685–695.
Pasternak, C., Ton-Hoang, B., Coste, G., Bailone, A., Chandler, M., and Sommer, S. (2010) Irradiation-induced Deinococcus radiodurans genome fragmentation triggers transposition of a single resident insertion sequence. *PLoS Genet* **6**: e1000799.
Pazy, Y., Wollish, A.C., Thomas, S.A., Miller, P.J., Collins, E.J., Bourret, R.B., and Silversmith, R.E. (2009) Matching biochemical reaction kinetics to the timescales of life: structural determinants that influence the autodephosphorylation rate of response regulator proteins. *J Mol Biol* **392**: 1205–1220.
Pato, M.L., and Reich, C. (1982) Instability of transposase activity: evidence from bacteriophage mu DNA replication. *Cell* **29**: 219–225.
Pato, M.L., and Banerjee, M. (1996) The Mu strong gyrase-binding site promotes efficient synapsis of the prophage termini. *Mol Microbiol* **22**: 283–292.

Pato, M.L. (2004) Replication of Mu prophages lacking the central strong gyrase site. *Res Microbiol* **155**: 553–558.

Paul, R., Abel, S., Wassmann, P., Beck, A., Heerklotz, H., and Jenal, U. (2007) Activation of the diguanylate cyclase PleD by phosphorylation-mediated dimerization. *J Biol Chem* **282**: 29170–29177.

Paulsen, I.T., Press, C.M., Ravel, J., Kobayashi, D.Y., Myers, G.S., Mavrodi, D.V., DeBoy, R.T., Seshadri, R., Ren, Q., Madupu, R., Dodson, R.J., Durkin, A.S., Brinkac, L.M., Daugherty, S.C., Sullivan, S.A., Rosovitz, M.J., Gwinn, M.L., Zhou, L., Schneider, D.J., Cartinhour, S.W., Nelson, W.C., Weidman, J., Watkins, K., Tran, K., Khouri, H., Pierson, E.A., Pierson, L.S., 3rd, Thomashow, L.S., and Loper, J.E. (2005) Complete genome sequence of the plant commensal Pseudomonas fluorescens Pf-5. *Nat Biotechnol* **23**: 873–878.

Peix, A., Ramirez-Bahena, M.H., and Velazquez, E. (2009) Historical evolution and current status of the taxonomy of genus Pseudomonas. *Infect Genet Evol* **9**: 1132–1147.

Perez, J.C., Shin, D., Zwir, I., Latifi, T., Hadley, T.J., and Groisman, E.A. (2009) Evolution of a bacterial regulon controlling virulence and Mg(2+) homeostasis. *PLoS Genet* **5**: e1000428.

Peters, J.E., and Craig, N.L. (2000) Tn7 transposes proximal to DNA double-strand breaks and into regions where chromosomal DNA replication terminates. *Mol Cell* **6**: 573–582.

Peters, J.E., and Craig, N.L. (2001) Tn7 recognizes transposition target structures associated with DNA replication using the DNA-binding protein TnsE. *Genes Dev* **15**: 737–747.

Peters, N.K., and Verma, D.P. (1990) Phenolic compounds as regulators of gene expression in plant-microbe relations. *Mol Plant Microbe Interact* **3**: 4–8.

Polard, P., and Chandler, M. (1995) Bacterial transposases and retroviral integrases. *Mol Microbiol* **15**: 13–23.

Pratt, L.A., Hsing, W., Gibson, K.E., and Silhavy, T.J. (1996) From acids to *osmZ*: multiple factors influence synthesis of the OmpF and OmpC porins in *Escherichia coli*. *Mol Microbiol* **20**: 911–917.

Pratt, L.A., and Silhavy, T.J. (1996) The response regulator SprE controls the stability of RpoS. *Proc Natl Acad Sci U S A.* **93**: 2488–2492.

Prost, L.R., Daley, M.E., Le Sage, V., Bader, M.W., Le Moual, H., Klevit, R.E., and Miller, S.I. (2007) Activation of the bacterial sensor kinase PhoQ by acidic pH. *Mol Cell* **26**: 165–174.

Purcell, E.B., Siegal-Gaskins, D., Rawling, D.C., Fiebig, A., and Crosson, S. (2007) A photosensory two-component system regulates bacterial cell attachment. *Proc Natl Acad Sci U S A* **104**: 18241–18246.

Putrinš, M., Ilves, H., Kivisaar, M., and Hõrak, R. (2008) ColRS two-component system prevents lysis of subpopulation of glucose-grown *Pseudomonas putida*. *Environ Microbiol* **10**: 2886–2893.

Putrinš, M., Ainelo, A., and Hõrak, R. (2010a) ColRS system is essential for the viability of hungry subpopulation of glucose-growing *Pseudomonas putida*. Manuscript.

Putrinš, M., Ilves, H., Lilje, L., Kivisaar, M., and Hõrak, R. (2010b) The impact of ColRS two-component system and TtgABC efflux pump on phenol tolerance of *Pseudomonas putida* becomes evident only in growing bacteria. *BMC Microbiol* **10**: 110.

Qian, Z., Wang, Q., Tong, W., Zhou, C., Wang, Q., and Liu, S. (2010) Regulation of galactose metabolism through the HisK:GalR two-component system in Thermoanaerobacter tengcongensis. *J Bacteriol* **192**: 4311–4316.

Rahme, L.G., Ausubel, F.M., Cao, H., Drenkard, E., Goumnerov, B.C., Lau, G.W., Mahajan-Miklos, S., Plotnikova, J., Tan, M.W., Tsongalis, J., Walendziewicz, C.L., and Tompkins, R.G. (2000) Plants and animals share functionally common bacterial virulence factors. *Proc Natl Acad Sci U S A* **97**: 8815–8821.

Ramos-Gonzalez, M.I., Campos, M.J., and Ramos, J.L. (2005) Analysis of *Pseudomonas putida* KT2440 gene expression in the maize rhizosphere: in vitro expression technology capture and identification of root-activated promoters. *J Bacteriol* **187**: 4033–4041.

Ramos, J.L., Wasserfallen, A., Rose, K., and Timmis, K.N. (1987) Redesigning metabolic routes: manipulation of TOL plasmid pathway for catabolism of alkylbenzoates. *Science* **235**: 593–596.

Rao, J.E., Miller, P.S., and Craig, N.L. (2000) Recognition of triple-helical DNA structures by transposon Tn7. *Proc Natl Acad Sci U S A* **97**: 3936–3941.

Rasis, M., and Segal, G. (2009) The LetA-RsmYZ-CsrA regulatory cascade, together with RpoS and PmrA, post-transcriptionally regulates stationary phase activation of Legionella pneumophila Icm/Dot effectors. *Mol Microbiol* **72**: 995–1010.

Regenhardt, D., Heuer, H., Heim, S., Fernandez, D.U., Strompl, C., Moore, E.R., and Timmis, K.N. (2002) Pedigree and taxonomic credentials of Pseudomonas putida strain KT2440. *Environ Microbiol* **4**: 912–915.

Reimmann, C., Moore, R., Little, S., Savioz, A., Willetts, N.S., and Haas, D. (1989) Genetic structure, function and regulation of the transposable element IS21. *Mol Gen Genet* **215**: 416–424.

Rhee, J.E., Sheng, W., Morgan, L.K., Nolet, R., Liao, X., and Kenney, L.J. (2008) Amino acids important for DNA recognition by the response regulator OmpR. *J Biol Chem* **283**: 8664–8677.

Roberts, A.P., and Mullany, P. (2009) A modular master on the move: the Tn916 family of mobile genetic elements. *Trends Microbiol* **17**: 251–258.

Roberts, D., Hoopes, B.C., McClure, W.R., and Kleckner, N. (1985) IS*10* transposition is regulated by DNA adenine methylation. *Cell* **43**: 117–130.

Robinson, V.L., Buckler, D.R., and Stock, A.M. (2000) A tale of two components: a novel kinase and a regulatory switch. *Nat Struct Biol* **7**: 626–633.

Rodrigue, A., Quentin, Y., Lazdunski, A., Mejean, V., and Foglino, M. (2000) Two-component systems in *Pseudomonas aeruginosa*: why so many? *Trends Microbiol* **8**: 498–504.

Roma-Rodrigues, C., Santos, P.M., Benndorf, D., Rapp, E., and Sa-Correia, I. (2010) Response of Pseudomonas putida KT2440 to phenol at the level of membrane proteome. *J Proteomics* **73**: 1461–1478.

Rood, J.I. (1998) Virulence genes of Clostridium perfringens. *Annu Rev Microbiol* **52**: 333–360.

Ross, P., Weinhouse, H., Aloni, Y., Michaeli, D., Weinberger-Ohana, P., Mayer, R., Braun, S., de Vroom, E., van der Marel, G.A., van Boom, J.H., and Benziman, M. (1987) Regulation of cellulose synthesis in *Acetobacter xylinum* by cyclic diguanylic acid. *Nature* **325**: 279–281.

Ross, P., Mayer, R., and Benziman, M. (1991) Cellulose biosynthesis and function in bacteria. *Microbiol Rev* **55**: 35–58.

Rowley, G., Spector, M., Kormanec, J., and Roberts, M. (2006) Pushing the envelope: extracytoplasmic stress responses in bacterial pathogens. *Nat Rev Microbiol* **4**: 383–394.

Ruiz, N., and Silhavy, T.J. (2005) Sensing external stress: watchdogs of the *Escherichia coli* cell envelope. *Curr Opin Microbiol* **8**: 122–126.

Ryder, C., Byrd, M., and Wozniak, D.J. (2007) Role of polysaccharides in Pseudomonas aeruginosa biofilm development. *Curr Opin Microbiol* **10**: 644–648.

Sahr, T., Bruggemann, H., Jules, M., Lomma, M., Albert-Weissenberger, C., Cazalet, C., and Buchrieser, C. (2009) Two small ncRNAs jointly govern virulence and transmission in Legionella pneumophila. *Mol Microbiol* **72**: 741–62.

Sanders, D.A., Gillece-Castro, B.L., Stock, A.M., Burlingame, A.L., and Koshland, D.E., Jr. (1989) Identification of the site of phosphorylation of the chemotaxis response regulator protein, CheY. *J Biol Chem* **264**: 21770–21778.

Santos, P.M., Benndorf, D., and Sa-Correia, I. (2004) Insights into *Pseudomonas putida* KT2440 response to phenol-induced stress by quantitative proteomics. *Proteomics* **4**: 2640–2652.

Schmidt, K.D., Tummler, B., and Romling, U. (1996) Comparative genome mapping of Pseudomonas aeruginosa PAO with P. aeruginosa C, which belongs to a major clone in cystic fibrosis patients and aquatic habitats. *J Bacteriol* **178**: 85–93.

Schneider, D., and Lenski, R.E. (2004) Dynamics of insertion sequence elements during experimental evolution of bacteria. *Res Microbiol* **155**: 319–327.

Schobert, M., and Tielen, P. (2010) Contribution of oxygen-limiting conditions to persistent infection of Pseudomonas aeruginosa. *Future Microbiol* **5**: 603–621.

Schweizer, H.P. (2003) Efflux as a mechanism of resistance to antimicrobials in Pseudomonas aeruginosa and related bacteria: unanswered questions. *Genet Mol Res* **2**: 48–62.

Seringhaus, M., Kumar, A., Hartigan, J., Snyder, M., and Gerstein, M. (2006) Genomic analysis of insertion behavior and target specificity of mini-Tn7 and Tn3 transposons in *Saccharomyces cerevisiae*. *Nucleic Acids Res* **34**: e57.

Sherratt, D. (1989) Tn*3* and Related Transposable Elements: Site-Specific Recombination and Transposition. In *Mobile DNA*. Berg, D.E. and Howe, M.M. (eds). Washington, D. C.: American Society for Microbiology, pp. 163–184.

Shin, S., and Park, C. (1995) Modulation of flagellar expression in *Escherichia coli* by acetyl phosphate and the osmoregulator OmpR. *J Bacteriol* **177**: 4696–4702.

Shu, C.J., and Zhulin, I.B. (2002) ANTAR: an RNA-binding domain in transcription antitermination regulatory proteins. *Trends Biochem Sci.* **27**: 3–5.

Sidote, D.J., Barbieri, C.M., Wu, T., and Stock, A.M. (2008) Structure of the Staphylococcus aureus AgrA LytTR domain bound to DNA reveals a beta fold with an unusual mode of binding. *Structure* **16**: 727–735.

Siguier, P., Filee, J., and Chandler, M. (2006) Insertion sequences in prokaryotic genomes. *Curr Opin Microbiol* **9**: 526–531.

Sikkema, J., de Bont, J.A., and Poolman, B. (1994) Interactions of cyclic hydrocarbons with biological membranes. *J Biol Chem* **269**: 8022–8028.

Sikkema, J., de Bont, J.A., and Poolman, B. (1995) Mechanisms of membrane toxicity of hydrocarbons. *Microbiol Rev* **59**: 201–222.

Silhavy, T.J., Kahne, D., and Walker, S. (2010) The bacterial cell envelope. *Cold Spring Harb Perspect Biol* **2**: a000414.

Simons, R.W., Hoopes, B.C., McClure, W.R., and Kleckner, N. (1983) Three promoters near the termini of IS*10*: pIN, pOUT, and pIII. *Cell* **34**: 673–682.

Simons, R.W., and Kleckner, N. (1983) Translational control of IS*10* transposition. *Cell* **34**: 683–691.

Skelding, Z., Sarnovsky, R., and Craig, N.L. (2002) Formation of a nucleoprotein complex containing Tn*7* and its target DNA regulates transposition initiation. *Embo J* **21**: 3494–3504.

Skerker, J.M., Prasol, M.S., Perchuk, B.S., Biondi, E.G., and Laub, M.T. (2005) Two-component signal transduction pathways regulating growth and cell cycle progression in a bacterium: a system-level analysis. *PLoS Biol* **3**: e334.

Smith, R.L., Kaczmarek, M.T., Kucharski, L.M., and Maguire, M.E. (1998) Magnesium transport in Salmonella typhimurium: regulation of mgtA and mgtCB during invasion of epithelial and macrophage cells. *Microbiology* **144 (Pt 7)**: 1835–1843.

Soncini, F.C., and Groisman, E.A. (1996) Two-component regulatory systems can interact to process multiple environmental signals. *J Bacteriol* **178**: 6796–6801.

Sonenshein, A.L. (2000) Control of sporulation initiation in Bacillus subtilis. *Curr Opin Microbiol* **3**: 561–566.

Spiers, A.J., Buckling, A., and Rainey, P.B. (2000) The causes of *Pseudomonas* diversity. *Microbiology* **146**: 2345–2350.

Stanley, D., Fraser, S., Stanley, G.A., and Chambers, P.J. (2010) Retrotransposon expression in ethanol-stressed Saccharomyces cerevisiae. *Appl Microbiol Biotechnol* **87**: 1447–1454.

Stephenson, K., and Hoch, J.A. (2004) Developing inhibitors to selectively target two-component and phosphorelay signal transduction systems of pathogenic micro-organisms. *Curr Med Chem* **11**: 765–773.

Stock, A.M., Mottonen, J.M., Stock, J.B., and Schutt, C.E. (1989) Three-dimensional structure of CheY, the response regulator of bacterial chemotaxis. *Nature* **337**: 745–749.

Stock, J., and Da Re, S. (2000) Signal transduction: response regulators on and off. *Curr Biol* **10**: R420–424.

Swem, L.R., Gong, X., Yu, C.A., and Bauer, C.E. (2006) Identification of a ubiquinone-binding site that affects autophosphorylation of the sensor kinase RegB. *J Biol Chem* **281**: 6768–6775.

Swingle, B., O'Carroll, M., Haniford, D., and Derbyshire, K.M. (2004) The effect of host-encoded nucleoid proteins on transposition: H-NS influences targeting of both IS*903* and Tn*10*. *Mol Microbiol* **52**: 1055–1067.

Zerbib, D., Polard, P., Escoubas, J.M., Galas, D., and Chandler, M. (1990) The regulatory role of the IS*1*-encoded InsA protein in transposition. *Mol Microbiol* **4**: 471–477.

Zhang, S.S., He, Y.Q., Xu, L.M., Chen, B.W., Jiang, B.L., Liao, J., Cao, J.R., Liu, D., Huang, Y.Q., Liang, X.X., Tang, D.J., Lu, G.T., and Tang, J.L. (2008) A putative *colR(XC1049)-colS(XC1050)* two-component signal transduction system in *Xanthomonas campestris* positively regulates *hrpC* and *hrpE* operons and is involved in virulence, the hypersensitive response and tolerance to various stresses. *Res Microbiol*. **159**: 569–78.

Zhang, Y.M., and Rock, C.O. (2008) Membrane lipid homeostasis in bacteria. *Nat Rev Microbiol* **6**: 222–233.

Zhao, R., Collins, E.J., Bourret, R.B., and Silversmith, R.E. (2002) Structure and catalytic mechanism of the E. coli chemotaxis phosphatase CheZ. *Nat Struct Biol* **9**: 570–575.

Zhu, Y., Qin, L., Yoshida, T., and Inouye, M. (2000) Phosphatase activity of histidine kinase EnvZ without kinase catalytic domain. *Proc Natl Acad Sci U S A* **97**: 7808–7813.

Zuber, S., Carruthers, F., Keel, C., Mattart, A., Blumer, C., Pessi, G., Gigot-Bonnefoy, C., Schnider-Keel, U., Heeb, S., Reimmann, C., and Haas, D. (2003) GacS sensor domains pertinent to the regulation of exoproduct formation and to the biocontrol potential of Pseudomonas fluorescens CHA0. *Mol Plant Microbe Interact* **16**: 634–644.

Tanaka, T., Saha, S.K., Tomomori, C., Ishima, R., Liu, D., Tong, K.I., Park, H., Dutta, R., Qin, L., Swindells, M.B., Yamazaki, T., Ono, A.M., Kainosho, M., Inouye, M., and Ikura, M. (1998) NMR structure of the histidine kinase domain of the *E. coli* osmosensor EnvZ. *Nature* **396**: 88–92.

Teesalu, M. (2008) The two-component signal transduction system ColRS is necessary in Pseudomonas putida when grown on glucose in magnesium limiting conditions. In *LOTE*. Vol. BSc Tartu: University of Tartu.

Teplitski, M., Goodier, R.I., and Ahmer, B.M. (2003) Pathways leading from BarA/SirA to motility and virulence gene expression in *Salmonella*. *J Bacteriol* **185**: 7257–7265.

Teplitski, M., Al-Agely, A., and Ahmer, B.M. (2006) Contribution of the SirA regulon to biofilm formation in Salmonella enterica serovar Typhimurium. *Microbiology* **152**: 3411–3424.

Teras, R., Hõrak, R., and Kivisaar, M. (2000) Transcription from fusion promoters generated during transposition of transposon Tn*4652* is positively affected by integration host factor in *Pseudomonas putida*. *J Bacteriol* **182**: 589–598.

Teras, R., Jakovleva, J., and Kivisaar, M. (2009) Fis negatively affects binding of Tn4652 transposase by out-competing IHF from the left end of Tn4652. *Microbiology* **155**: 1203–1214.

Thomason, P.A., Traynor, D., Cavet, G., Chang, W.T., Harwood, A.J., and Kay, R.R. (1998) An intersection of the cAMP/PKA and two-component signal transduction systems in Dictyostelium. *Embo J* **17**: 2838–2845.

Timmis, K.N. (2002) Pseudomonas putida: a cosmopolitan opportunist par excellence. *Environ Microbiol* **4**: 779–781.

Tobes, R., and Pareja, E. (2006) Bacterial repetitive extragenic palindromic sequences are DNA targets for Insertion Sequence elements. *BMC Genomics* **7**: 62.

Tomenius, H., Pernestig, A.K., Jonas, K., Georgellis, D., Mollby, R., Normark, S., and Melefors, O. (2006) The Escherichia coli BarA-UvrY two-component system is a virulence determinant in the urinary tract. *BMC Microbiol* **6**: 27.

Tomomori, C., Tanaka, T., Dutta, R., Park, H., Saha, S.K., Zhu, Y., Ishima, R., Liu, D., Tong, K.I., Kurokawa, H., Qian, H., Inouye, M., and Ikura, M. (1999) Solution structure of the homodimeric core domain of *Escherichia coli* histidine kinase EnvZ. *Nat Struct Biol* **6**: 729–734.

Toro-Roman, A., Mack, T.R., and Stock, A.M. (2005) Structural analysis and solution studies of the activated regulatory domain of the response regulator ArcA: a symmetric dimer mediated by the alpha4-beta5-alpha5 face. *J Mol Biol* **349**: 11–26.

Trent, M.S., Pabich, W., Raetz, C.R., and Miller, S.I. (2001) A PhoP/PhoQ-induced Lipase (PagL) that catalyzes 3-O-deacylation of lipid A precursors in membranes of *Salmonella typhimurium*. *J Biol Chem* **276**: 9083–9092.

Tsuda, M., and Iino, T. (1987) Genetic analysis of a transposon carrying toluene degrading genes on a TOL plasmid pWW0. *Mol Gen Genet* **210**: 270–276.

Tsuda, M., Minegishi, K., and Iino, T. (1989) Toluene transposons Tn4651 and Tn4653 are class II transposons. *J Bacteriol* **171**: 1386–1393.
Tzeng, Y.L., Zhou, X., Bao, S., Zhao, S., Noble, C., and Stephens, D.S. (2006) Autoregulation of the MisR/MisS two-component signal transduction system in Neisseria meningitidis. *J Bacteriol* **188**: 5055–5065.
Ulrich, L.E., Koonin, E.V., and Zhulin, I.B. (2005) One-component systems dominate signal transduction in prokaryotes. *Trends Microbiol* **13**: 52–56.
Vallet-Gely, I., Novikov, A., Augusto, L., Liehl, P., Bolbach, G., Pechy-Tarr, M., Cosson, P., Keel, C., Caroff, M., and Lemaitre, B. (2010) Association of hemolytic activity of Pseudomonas entomophila, a versatile soil bacterium, with cyclic lipopeptide production. *Appl Environ Microbiol* **76**: 910–921.
Valls, M., Buckle, M., and de Lorenzo, V. (2002) *In vivo* UV laser footprinting of the *Pseudomonas putida* sigma 54 Pu promoter reveals that integration host factor couples transcriptional activity to growth phase. *J Biol Chem* **277**: 2169–2175.
Valverde, C., and Haas, D. (2008) Small RNAs controlled by two-component systems. *Adv Exp Med Biol* **631**: 54–79.
Wang, X., and Higgins, N.P. (1994) 'Muprints' of the *lac* operon demonstrate physiological control over the randomness of *in vivo* transposition. *Mol Microbiol* **12**: 665–677.
Wang, X., Dubey, A.K., Suzuki, K., Baker, C.S., Babitzke, P., and Romeo, T. (2005) CsrA post-transcriptionally represses pgaABCD, responsible for synthesis of a biofilm polysaccharide adhesin of Escherichia coli. *Mol Microbiol* **56**: 1648–1663.
Wardle, S.J., O'Carroll, M., Derbyshire, K.M., and Haniford, D.B. (2005) The global regulator H-NS acts directly on the transpososome to promote Tn10 transposition. *Genes Dev* **19**: 2224–2235.
Wardle, S.J., Chan, A., and Haniford, D.B. (2009) H-NS binds with high affinity to the Tn10 transpososome and promotes transpososome stabilization. *Nucleic Acids Res* **37**: 6148–6160.
Varughese, K.I. (2002) Molecular recognition of bacterial phosphorelay proteins. *Curr Opin Microbiol.* **5**: 142–148.
Wassmann, P., Chan, C., Paul, R., Beck, A., Heerklotz, H., Jenal, U., and Schirmer, T. (2007) Structure of BeF3-modified response regulator PleD: implications for diguanylate cyclase activation, catalysis, and feedback inhibition. *Structure* **15**: 915–927.
Watanabe, T., Okada, A., Gotoh, Y., and Utsumi, R. (2008) Inhibitors targeting two-component signal transduction. *Adv Exp Med Biol* **631**: 229–236.
Weinreich, M.D., and Reznikoff, W.S. (1992) Fis plays a role in Tn*5* and IS*50* transposition. *J Bacteriol* **174**: 4530–4537.
Vescovi, E.G., Ayala, Y.M., Di Cera, E., and Groisman, E.A. (1997) Characterization of the bacterial sensor protein PhoQ. Evidence for distinct binding sites for Mg2+ and Ca2+. *J Biol Chem* **272**: 1440–1443.
West, A.H., and Stock, A.M. (2001) Histidine kinases and response regulator proteins in two-component signaling systems. *Trends Biochem Sci* **26**: 369–376.
Whistler, C.A., and Ruby, E.G. (2003) GacA regulates symbiotic colonization traits of *Vibrio fischeri* and facilitates a beneficial association with an animal host. *J Bacteriol* **185**: 7202–7212.
Whistler, C.A., Koropatnick, T.A., Pollack, A., McFall-Ngai, M.J., and Ruby, E.G. (2007) The GacA global regulator of *Vibrio fischeri* is required for normal host tissue responses that limit subsequent bacterial colonization. *Cell Microbiol* **9**: 766–778.

Whitfield, C.R., Wardle, S.J., and Haniford, D.B. (2009) The global bacterial regulator H-NS promotes transpososome formation and transposition in the Tn5 system. *Nucleic Acids Res* **37**: 309–321.

Wiater, L.A., and Grindley, N.D. (1988) Gamma delta transposase and integration host factor bind cooperatively at both ends of gamma delta. *Embo J* **7**: 1907–1911.

Wiater, L.A., and Grindley, N.D. (1990) Uncoupling of transpositional immunity from gamma delta transposition by a mutation at the end of gamma delta. *J Bacteriol* **172**: 4959–4963.

Vidal, O., Longin, R., Prigent-Combaret, C., Dorel, C., Hooreman, M., and Lejeune, P. (1998) Isolation of an *Escherichia coli* K-12 mutant strain able to form biofilms on inert surfaces: involvement of a new *ompR* allele that increases curli expression. *J Bacteriol* **180**: 2442–2449.

Vodovar, N., Vallenet, D., Cruveiller, S., Rouy, Z., Barbe, V., Acosta, C., Cattolico, L., Jubin, C., Lajus, A., Segurens, B., Vacherie, B., Wincker, P., Weissenbach, J., Lemaitre, B., Medigue, C., and Boccard, F. (2006) Complete genome sequence of the entomopathogenic and metabolically versatile soil bacterium *Pseudomonas entomophila*. *Nat Biotechnol* **24**: 673–679.

Volz, K., and Matsumura, P. (1991) Crystal structure of *Escherichia coli* CheY refined at 1.7-A resolution. *J Biol Chem* **266**: 15511–15519.

Won, H.S., Lee, Y.S., Lee, S.H., and Lee, B.J. (2009) Structural overview on the allosteric activation of cyclic AMP receptor protein. *Biochim Biophys Acta* **1794**: 1299–1308.

Wu, X., Monchy, S., Taghavi, S., Zhu, W., Ramos, J., and van der Lelie, D. (2010) Comparative genomics and functional analysis of niche-specific adaptation in Pseudomonas putida. *FEMS Microbiol Rev* no. doi: 10.1111/j.1574–6976.2010. 00249.x

Wösten, M.M., Kox, L.F., Chamnongpol, S., Soncini, F.C., and Groisman, E.A. (2000) A signal transduction system that responds to extracellular iron. *Cell* **103**: 113–125.

Yamamoto, K., Hirao, K., Oshima, T., Aiba, H., Utsumi, R., and Ishihama, A. (2005) Functional characterization in vitro of all two-component signal transduction systems from Escherichia coli. *J Biol Chem* **280**: 1448–1456.

Yanagihara, K., and Mizuuchi, K. (2002) Mismatch-targeted transposition of Mu: a new strategy to map genetic polymorphism. *Proc Natl Acad Sci U S A* **99**: 11317–11321.

Yigit, H., and Reznikoff, W.S. (1999) *Escherichia coli* DNA topoisomerase I copurifies with Tn*5* transposase, and Tn*5* transposase inhibits topoisomerase I. *J Bacteriol* **181**: 3185–3192.

Yin, J.C., Krebs, M.P., and Reznikoff, W.S. (1988) Effect of dam methylation on Tn*5* transposition. *J Mol Biol* **199**: 35–45.

Yoshida, T., Qin, L., Egger, L.A., and Inouye, M. (2006) Transcription regulation of *ompF* and *ompC* by a single transcription factor, OmpR. *J Biol Chem* **17**: 17.

Yu, J., Penaloza-Vazquez, A., Chakrabarty, A.M., and Bender, C.L. (1999) Involvement of the exopolysaccharide alginate in the virulence and epiphytic fitness of Pseudomonas syringae pv. syringae. *Mol Microbiol* **33**: 712–720.

Yuste, L., Hervas, A.B., Canosa, I., Tobes, R., Jimenez, J.I., Nogales, J., Perez-Perez, M.M., Santero, E., Diaz, E., Ramos, J.L., de Lorenzo, V., and Rojo, F. (2006) Growth phase-dependent expression of the *Pseudomonas putida* KT2440 transcriptional machinery analysed with a genome-wide DNA microarray. *Environ Microbiol* **8**: 165–177.

SUMMARY IN ESTONIAN

Kahekomponentse signaaliraja ColR-ColS ja mobiilse elemendi Tn*4652* osalus *Pseudomonas putida* kohastumises

Bakterid on silmale nähtamatud ainuraksed organismid, kes asustavad kõikvõimlikke elupaiku. Mikroobid suudavad ellu jääda ka nii äärmuslike tingimustega kohtades nagu kuumaveeallikad, ookeani süvikud, polaarjää jne. Looduslikes elupaikades võivad tingimused kiirelt vahelduda ja bakterid peavad ellujäämise nimel kohanema, mis võib toimuda läbi füsioloogiliste või geneetiliste muutuste. Bakteriraku füsioloogias toimuvaid muutusi reguleerivad mitmesugused signaalsüsteemid. Kõige tavalisem signaalsüsteem koosneb kahest valgust: keskkonda tajuvast sensorvalgust raku membraanis ja tema partnerist, mis reguleerib vastusena signaalile geeniekspressiooni. Geneetiline ehk evolutsiooniline kohandumine uute tingimustega on pikaajalisem protsess, kuna vajab muutusi organismi genoomis ja on seeläbi ka järgastele edasi pärandatav. Genoomse DNA muutused võivad tekkida juhuslike punktmutatsioonide või suuremate ümberkorraldustena. Ulatuslike DNA ümberkorralduste allikaks võivad olla ka transposoonid ehk liikuvad DNA elemendid, mis võimaldavad geneetilise informatsiooni vahetamist ka organismide vahel.

Bakteriperekond *Pseudomonas* on tuntud oma hea kohanemisvõime poolest, mis laseb neil asustada erinevaid keskkondi nagu muld, vesi, taimed, loomad ja inimene. Perekonna *Pseudomonas* liikmed suudavad hästi kohaneda ka antibiootikumidega ja nende antibiootikumi resistentsus tekitab pidevalt probleeme nüüdisaegses meditsiinis. Käesolevas töös olen ma uurinud keskkonnabakteri *Pseudomonas putida* kahekomponentse signaalsüsteemi ColR-ColS osa bakteri kohanemises. Antud signaalsüsteemi on seostatud mikroobi võimega koloniseerida taimejuuri, virulentsusega ja transposooni Tn*4652* transpositsiooniga (Dekkers *et al.*, 1998; Garvis *et al.*, 2009; Hõrak *et al.*, 2004), kuid tema täpne funktsioon on jäänud selgusetuks. Seetõttu on minu töö olnud suunatud ColR-ColS signaaliraja funktsiooni mõistmisele ja selleks püüdsin välja selgitada milliseid geene vastuse regulaator ColR mõjutab. Töö tulemused võib kokku võtta järgnevalt:

- ColR-ColS signaalsüsteem mõjutab Tn*3* perekonna transposooni Tn*4652* transpositsiooni kaudselt.
  - Rikutud ColR-ColS signaalirajaga *P. putida* rakkudes vähenes Tn*4652* transpositsiooni sagedus suurusjärgu võrra (Hõrak *et al.*, 2004). See tulemus oli mõõdetud fenoolil nälgivas populatsioonis, kus Tn*4652* transpositsiooni tagajärjel tekkisid fenoolil kasvada suutvad kolooniad. Minu tööst selgus, et Tn*4652* transpositsioon sõltus katkestatud ColR-ColS signaalirajaga rakkudes pöördvõrdeliselt fenooli kontsentratsioonist – mida rohkem oli söötmes fenooli, seda väiksem oli transpositsiooni sagedus. Selline seos andis alust arvata, et *P. putida* rakud on katkestatud

ColR-ColS signaaliraja korral fenooli suhtes tundlikumad ja fenooli toksiline efekt bakterirakule võiks põhjustada Tn*4652* transpositsiooni vähenemise.

– Uurimaks Tn*4652* transpositsiooni fenoolist sõltumatutes tingimustes disainisin uue transpositsiooni testsüsteemi. Kuna uue testsüsteemi puhul polnud Tn*4652* transpositsioon ColR-ColS signaaliraja puudumisest mõjutatud, siis kinnitasid need tulemused meie hüpoteesi, et fenooli toksiline mõju pärsib Tn*4652* transpositsiooni *P. putida* rakkudes ColR puudumisel.
– Selektsioonist sõltumatu testsüsteem võimaldas uurida transposooni Tn*4652* märklaua valiku strateegiat. Kuna valdav enamus transposoon Tn*4652* tuvastatud insertsioonikohtadest olid unikaalsed, siis pole Tn*4652* transpositsioon piiratud märklaua valikuga. Siiski oli Tn*4652* märklaudadest võimalik moodustada tugev konsensusjärjestus, mis sarnaneb transposoon Tn*3* märklauale ja lubab järeldada, et selle perekonna elementide märklaua valik ei ole päris juhuslik.

Suurem osa mu tööst keskendus ColR-ColS signaaliraja funktsioonide uurimisele *P. putida* rakkudes ja selle tulemused võib kokku võtta järgnevalt:

- ColR-ColS signaaliraja peamiseks märklauaks on bakteriraku membraan.
  – Suur osa vastuse regulaator ColR kontrollitavatest geenidest kodeerib membraaniga või membraani sünteesiga seotud valke. ColR märklaud-geenide hulgas on sisemises ja välimises membraanis paiknevaid valke kodeerivaid geene, aga ka geene, mis osalevad lipiidide ja rasvhapete sünteesis või modifikatsioonis. Samuti on ColR-ColS signaalsüsteemi poolt reguleeritud alginaadi ja tselluloosi hulk, mis moodustab rakku ümbritseva kapsli ning rakuseina peamise komponendi peptiidoglükaani süntees.
- Vastuse regulaatorvalk ColR seondub DNA järjestustele, mis sarnanevad ülejäänud OmpR perekonna valkude poolt äratuntavatele kordusjärjestustele. Sellised seondumisjärjestused lubavad oletada, et ColR valk seondub DNA heeliksga dimeerina.

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PUBLICATIONS

CURRICULUM VITAE

I. General data

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II. Scientific activities

Main research interests:

- The function and target genes of ColRS two-component system in environmental bacterium *Pseudomonas putida*.
- Target site selection criteria of Tn4652 transposon in soil bacterium *P. putida.*

List of publications:

1. Kivistik PA, Kivi R, Kivisaar M, Hõrak R. Identification of ColR binding consensus and prediction of regulon of ColRS two-component system. BMC Mol Biol. 2009 10:46.
2. Kivistik PA, Kivisaar M, Hõrak R. Target site selection of *Pseudomonas putida* transposon Tn*4652*. J Bacteriol. 2007 189: 3918–21.
3. Kivistik PA, Putrinš M, Püvi K, Ilves H, Kivisaar M, Hõrak R. The ColRS two-component system regulates membrane functions and protects *Pseudomonas putida* against phenol. J Bacteriol. 2006 188: 8109–17.

Fellowships and stipends:

1. Kristjan Jaak stipend 2006 ja 2010
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3. Lydia and Felix Krabi stipend 2004

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II. Teaduslik ja arendustegevus

Peamised uurimisvaldkonnad:

– Keskkonnabakteri *Pseudomonas putida* kahekomponentse signaalsüsteemi ColRS funktsiooni ja märklaudgeenide selgitamine.
– Keskkonnabakteri *P. putida* transposooni Tn*4652* märklaua valiku strateegia uurimine.

Publikatsioonide loetelu:

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2. Kivistik PA, Kivisaar M, Hõrak R. Target site selection of *Pseudomonas putida* transposon Tn*4652*. J Bacteriol. 2007 189: 3918–21.
3. Kivistik PA, Putrinš M, Püvi K, Ilves H, Kivisaar M, Hõrak R. The ColRS two-component system regulates membrane functions and protects *Pseudomonas putida* against phenol. J Bacteriol. 2006 188: 8109–17.

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