The LytS/LytTR-like histidine kinase/response regulator systems in *Escherichia coli* and their function in carbon source selectivity

Dissertation

der Fakultät für Biologie

der Ludwig-Maximilians-Universität München

vorgelegt von

Stefan Maximilian Behr

aus Gunzenhausen

München, Juli 2014

Gutachter:

1. Prof. Dr. Kirsten Jung
2. Prof. Dr. Thorsten Mascher

Datum der Abgabe: 29. Juli 2014

Datum der mündlichen Prüfung: 6. November 2014

Eidesstattliche Erklärung

Ich versichere hiermit an Eides statt, dass die vorgelegte Dissertation von mir selbstständig und ohne unerlaubte Hilfe angefertigt wurde. Des Weiteren erkläre ich, dass ich nicht anderweitig ohne Erfolg versucht habe, eine Dissertation einzureichen oder mich der Doktorprüfung zu unterziehen. Die folgende Dissertation liegt weder ganz, noch in wesentlichen Teilen einer anderen Prüfungskommission vor.

München,

Statuory Declaration

I declare that I have authored this thesis independently, that I have not used other than the declared sources/resources. As well I declare, that I have not submitted a dissertation without success and not passed the oral exam. The present dissertation (neither the entire dissertation nor parts) has not been presented to another examination board.

Munich,

Content

Nomenclature

Proteins are numbered according to the first methionine/valine in the wild type amino acid sequence designated as “1”. N- or C-terminal affinity tags are indicated corresponding to their position (e.g. 6His-YpdB or YpdB-6His).

Proteins containing amino acid substitutions are termed as follows: Based on the one-letter code the native amino acid is placed first, followed by its position, and the corresponding amino acid substitution (e.g. YpdB-D53E).

Deletions are marked with “Δ”.

Unless otherwise stated, nucleotide positions indicate the distance to the transcriptional start site (+1).

Abbreviations

ATP	adenosine- 5'-triphosphate
CA	catalytic and ATP binding domain
cAMP	cyclic adenosine-5'-monophosphate
c-di-GMP	bis-(3'-5')-cyclic dimeric guanosine monophosphate
cGMP	cyclic guanosine 5'-monophosphate
CM	cytoplasmic membrane
CP	cytoplasm
DHp	dimerization and histidine phosphotransfer domain
DNA	deoxyribonucleic acid
DNase	deoxyribonuclease
EMSA	electrophoretic mobility shift assay
GAF	protein domain present in cGMP-specific phosphodiesterases, adenylyl cyclases and FhlA proteins
HAMP	protein domain present in histidine kinases, adenylyl cyclases, methyl-accepting chemotaxis proteins, and some phosphatases
HK	histidine kinase
n-His tag	affinity tag composed of *n* histidine residues
HPt	histidine containing phosphotransfer protein
LB	lysogeny broth
MFS	major facilitator superfamily of transporters
RR	response regulator
PAGE	polyacrylamide gel electrophoresis
PAS	protein domain present in Per, Arnt, and Sim proteins
PP	periplasm
RNA	ribonucleic acid
RNase	ribonuclease
TM	transmembrane
TCS	two-component system
WT	wild type
w/v, v/v	weight per volume, volume per volume

Publications and Manuscripts Originating from this Thesis

CHAPTER 2

Kraxenberger, T.*, Fried, L.*, Behr, S., and Jung, K. (2012). First Insights into the Unexplored Two-Component System YehU/YehT in *Escherichia coli*. *J. Bacteriol.* 194(16):4272-4284.

CHAPTER 3

Fried, L.*, Behr, S.*, and Jung, K. (2013). Identification of a Target Gene and Activating Stimulus for the YpdA/YpdB Histidine Kinase/Response Regulator System in *Escherichia coli*. *J. Bacteriol.* 195(4):807-15.

CHAPTER 4

Behr, S.*, Fried, L.*, and Jung, K. (2014). Identification of a Novel Nutrient-Sensing Histidine Kinase/Response Regulator Network in *Escherichia coli*. *J. Bacteriol.* 196(11):2023-9.

CHAPTER 6

Behr, S., Heermann, R., and Jung, K. (2014). Manuscript: A novel allosteric DNA-binding mechanism of a bacterial response regulator. *Unpublished Manuscript.*

CHAPTER 7

Jung, K., Fried, L., Behr, S., and Heermann, R. (2012). Histidine kinases and response regulators in networks. *Curr. Opin. Microbiol.*15(2): 118-124.

Contributions to Publications and Manuscripts presented in this Thesis

CHAPTER 2

T. Kraxenberger, L. Fried, S. Behr, and K. Jung developed the concept of the study. T. Kraxenberger and L. Fried constructed all strains and plasmids and prepared the samples for the microarray analysis. L. Fried performed expression analysis via Northern blot. T. Kraxenberger and S. Behr determined YehT DNA-binding affinity and binding areas via EMSA, DNase I footprinting and expression analysis. L. Fried performed the initial *yjiY* expression screen and characterized the CRP binding site. T. Kraxenberger, L. Fried, S. Behr, and K. Jung wrote the manuscript.

CHAPTER 3

L. Fried, S. Behr, and K. Jung designed the concept of the study. L. Fried and S. Behr constructed all strains and plasmids and prepared the samples for the microarray analysis. L. Fried performed expression analysis via Northern blot. L. Fried and S. Behr determined YpdB DNA-binding affinity and binding areas via EMSA, DNase I footprinting and expression analysis. L. Fried performed the initial *yhjX* expression screen. L. Fried, S. Behr, and K. Jung wrote the manuscript.

CHAPTER 4

S. Behr, L. Fried, and K. Jung designed the concept of the study. S. Behr and L. Fried constructed all strains and plasmids. L. Fried performed expression analysis via qRT-PCR and revealed CsrA dependent regulation. S. Behr determined stimulus dependent crosswise alterations between both systems and described the influence of deletions in the signal transduction cascades. S. Behr performed in vivo protein-protein interaction studies. S. Behr, L. Fried, and K. Jung wrote the manuscript.

CHAPTER 6

S. Behr, R. Heermann, and K. Jung designed the concept of the study. S. Behr performed the protein affinity purification and size exclusion chromatography. R. Heermann and S. Behr did the surface plasmon resonance spectroscopy measurements. R. Heermann analyzed the resultant data (Concentration free calibration analysis (CFCA), Interaction Map technology (IM)). S. Behr and R. Heermann wrote the manuscript.

CHAPTER 7

K. Jung, L. Fried, S. Behr and R. Heermann designed and discussed the concept of the review and wrote sections 9 to 11. K. Jung wrote sections 6 and 7. L. Fried wrote section 3. S. Behr wrote sections 5 and 8 and R. Heermann wrote sections 1, 2 and 4.

We hereby confirm the above statements:

Stefan Behr

Prof. Dr. Kirsten Jung

Dr. Luitpold Fried

Dr. Tobias Kraxenberger

PD Dr. Ralf Heermann

Summary

Bacterial signal transduction systems continuously monitor internal and external parameters to enable an adaptive response to environmental changes. Two-component systems display the major group of bacterial signal transduction systems and consist of a sensor histidine kinase and cognate response regulator. Based on structural properties two-component systems can be classified into diverse protein families. Among these the widespread LytS/LytTR family of two-component systems regulates a variety of essential cellular processes in pathogenic Gram-positive bacteria, whereas only little is known about the corresponding systems in Gram-negatives.

The main focus of this thesis was to elucidate the function and interconnectivity of the two LytS/LytTR two-component systems YehU/YehT and YpdA/YpdB in the Gram-negative enterobacterium *Escherichia coli*. Based on bioinformatics, genetic and biochemical approaches this study provides new insights into signal perception, signal transduction and subsequent cellular adaptation for each system. Furthermore the coordinated regulatory interplay between both systems is described.

In chapter 2 the LytS/LytTR-like two-component system YehU/YehT was characterized. Based on transcriptome analysis, subsequent expression studies and gel retardation experiments *yjiY* was identified as the sole target gene of YehU/YehT. DNaseI footprints and nucleic acids substitution revealed YehT binding site, a direct repeat of ACC(G/A)CT(C/T)A separated by a 13 bp spacer within the *yjiY* promoter. Induction of *yjiY*, which encodes a putative inner membrane carbon starvation protein, was detected in media containing peptides and amino acids as carbon source. Furthermore, expression of *yjiY* was observed in the mid-exponential growth phase and was dependent on cAMP/CRP regulation.

The study of chapter 3 focused on the identification of YpdA/YpdB stimulus and target gene. Again using transcriptome analysis, subsequent expression studies and gel retardation experiments, *yhjX* was identified as sole target gene of YpdB. A direct repeat of GGCATTTCAT with 11 bp spacer within *yhjX* promoter determined the YpdB-binding site. Based on comprehensive *yhjX* expression analyses extracellular pyruvate was identified as potential stimulus. YhjX, a putative major facilitator superfamily transporter, was produced and shown to be membrane integrated.

In chapter 4 the nutrient-sensing signaling network of YehU/YehT and YpdA/YpdB was described. Both systems were activated at the transition to stationary phase. Stimulus dependent and mutational expression analyses revealed a regulatory interplay between both systems. In vivo interaction studies indicate the formation a large signaling unit between YehU/YpdA and their corresponding target proteins YjiY/YhjX. In addition carbon storage regulator A (CsrA) was shown to be involved in posttranscriptional regulation of both *yjiY* and *yhjX*.

In chapter 5 preliminary results of the putative accessory protein YehS in LytS/LytTR mediated gene expression of *Escherichia coli* are summarized. Deletion or overexpression of *yehS* revealed the same effect on target gene expression. Furthermore in vivo interaction studies identified interactions for YehS with both histidine kinases and response regulators. Together with initial structural studies a coupling function might be suggested.

In chapter 6 binding of YpdB to its corresponding promoter DNA was investigated. Mimicking phosphorylation in a constitutively active YpdB derivative demonstrated its need for proper DNA-binding in surface plasmon resonance spectroscopy measurements. Subsequent substitutions within the YpdB-binding site revealed two individual binding events in a cooperative fashion, which represents novel insights into LytTR mediated gene expression.

Finally, network formation in bacterial signal transduction systems is discussed. Different functions of accessory proteins in signal integration, scaffolding, interconnection and allosteric regulation, are described and illustrate the importance of coordinated signal transduction to control a multitude of in- and output processes.

Zusammenfassung

Bakterien beobachten eine Vielzahl interner und externer Parameter, um sich stets Veränderungen in ihrem Umfeld entsprechend anpassen zu können. Dabei stellen sogenannte Zwei-Komponenten-Systeme die größte Gruppe bakterielle Signaltransduktionssysteme dar. Sie bestehen aus einer Sensor-Histidinkinase und einem Antwortregulator. Auf Grund ihrer strukturellen Gemeinsamkeiten werden diese unterschiedlichen Proteinfamilien zugeordnet. Zwei-Komponenten-Systeme vom LytS/LytTR-Typ sind weit verbreitet und kontrollieren viele grundlegende Vorgänge in pathogenen Gram-positiven Organismen. Über die Regulation dieser Systeme in Gram-negativen Bakterien ist hingegen nur wenig bekannt.

Das Hauptaugenmerk dieser Arbeit lag in der Aufklärung der Funktion der beiden LytS/LytTR-artige Zwei-Komponenten-Systeme YehU/YehT und YpdA/YpdB in *Escherichia coli*. Basierend auf bioinformatischen, genetischen und biochemischen Methoden gewährt diese Arbeit neue Einblicke in die Zusammenhänge der Reizwahrnehmung, der Signaltransduktion und der daraus resultierenden Zellantwort beider System und beschreibt deren koordinierte Regulationsmechanismen.

In Kapitel 2 wurde das LytS/LytTR-artige Zwei-Komponenten-System YehU/YehT beschrieben. Mit Hilfe einer globalen Transkriptomanalyse, Expressionsstudien und Gelretardationsexperimente wurde *yjiY* als direktes Zielgen von YehT identifiziert. DNase-Schutz-Experimente und Nukleinsäure-Substitutionen im Promotor von *yjiY* ergaben eine YehT-Bindestelle mit der direkten Wiederholung der Sequenz ACC(G/A)CT(C/T)A getrennt durch ein 13 bp *spacer*-Motif. Die Expression des Gens *yjiY*, welches für ein putatives Transportprotein kodiert, konnte in Medien mit Aminosäuren und Peptiden als Kohlenstoffquelle beobachtet werden. Darüber hinaus wurde *yjiY* zum Ende der logarithmischen Wachstumsphase induziert und unterlag der Regulation durch cAMP/CRP.

In Kapitel 3 wurden Reiz und Zielgen von YpdA/YpdB untersucht. Mit Hilfe einer globalen Transkriptomanalyse, Expressionsstudien und Gelretardationsexperimente wurde *yhjX* als direktes Zielgen von YpdB identifiziert. Dabei stellt eine direkte Wiederholung der Sequenz GGCATTTCAT, getrennt durch ein 11 bp *spacer*-Motif, die YpdB-Bindestelle im *yhjX* Promotor dar. Mit Hilfe von Expressionsanalysen wurde extrazelluläres Pyruvat als möglicher Reiz für YpdA/YpdB identifiziert. Für YhjX, ein Protein der Major-Facilitator-Superfamilie, konnte Membranintegration nachgewiesen werden.

In Kapitel 4 wurde der Zusammenhang beider Systeme hinsichtlich des Vorkommens bestimmter Kohlenstoffquellen untersucht. Dabei wurde gezeigt, dass beide Systeme ihre Zielgene am Übergang in die stationäre Phase aktivieren. In Abhängigkeit des Reizes bzw. unter zu Hilfenahme von Gendeletionen wurde der Einfluss beider Systeme aufeinander untersucht. In vivo wurden Protein-Protein-Wechselwirkungen zwischen YehU/YpdA und den zugehörigen Transportproteinen YjiY/YhjX beobachtet. Dies legt die Notwendigkeit der Bildung eines größeren Signaltransduktions-Komplexes nahe. Darüber hinaus wurde der Einfluss von CsrA, einem globalen Kohlenstoffregulator, auf die posttranskriptionelle Regulation von *yjiY* und *yhjX* gezeigt.

In Kapitel 5 wurden die vorläufigen Ergebnisse eines möglicherweise akzessorischen Proteins YehS in der LytTR-vermittelten Signaltransduktion von *Escherichia coli* zusammengefasst. Dabei zeigten Deletion und Überexpression von *yehS* den gleichen Effekt auf die Zielgenexpression beider Systeme. In in vivo Interaktionsstudien wurden Protein-Protein-Wechselwirkungen zwischen YehS und den beiden Histidinkinasen als auch Antwortregulatorproteinen festgestellt. Zusammen mit ersten strukturellen Analysen ist eine Kopplungsfunktion (*coupling*) von YehS denkbar.

In Kapitel 6 wurde die Bindung des Antwortregulators YpdB an die zugehörige *yhjX* Promotor DNA untersucht. Mit Hilfe von Oberflächenplasmonresonanzspektroskopie wurde mit einem konstitutiv aktiven YpdB-Derivat die Notwendigkeit der Phosphorylierung für die DNA-Bindung gezeigt. Mit Hilfe verschiedener Nukleotidsubstitutionen in der YpdB DNA Bindestelle des *yhjX* Promotors konnte ein zweistufiger, kooperativer Bindemechanismus beobachtet werden, welcher neue Einblicke in die LytTR vermittelte Genexpression erlaubt.

Die letzte Studie dieser Arbeit beschreibt bis dato bekannte Verknüpfungsmöglichkeiten in der Signaltransduktion. Dabei werden insbesondere akzessorische Proteine sowie deren Funktionen bei der Signalintegration, dem *scaffolding* (Gerüstfunktion), dem *coupling* (Kopplung) und der allosterischen Regulation diskutiert. Um auf eine Vielzahl von Signalen entsprechend reagieren zu können, wird dabei anhand einiger Bespiele unter anderem die Notwendigkeit der koordinierten Regulation beschrieben.

1 Introduction

All living organisms are exposed to frequent fluctuations of environmental conditions within their natural habitats. Physical or chemical parameters, like temperature, oxygen content, pH, osmolarity or the availability of nutrients can change very fast and result in life threatening circumstances. Therefore unicellular organisms, in particular prokaryotes have evolved strategies to react and cope with different risks, like osmo- or pH-stress and carbon/nitrogen limitations. Besides environmental dangers it can also be beneficial to respond to other pro- or eukaryotic species. By synchronizing processes, like biofilm formation, bioluminescence or expression of host specific virulence factors bacteria can display multicellular behavior which plays an important role in co-evolutionary processes, like symbiosis or parasitism.

Bacterial signal transduction can be divided into three independent events: after recognition of an intra- or extracellular stimulus (I – signal perception) the signal is transferred to an effector protein (II – signal transmission). Its resultant activation induces cellular adaptations (III – adaptive cellular response).

In general three different mechanisms are known to achieve this function: σ-/anti-σ pairs, one-component systems and two-component systems (TCSs). In the predominant one-component systems signal recognition and activation of the effector is brought out within one single protein. Two-component systems in contrast display at least two functionally separated units: an often membrane-anchored sensory protein and a soluble effector counterpart.

1.1 Two-component systems

Two-component signaling genes are found in all three domains of life (Schaller *et al.*, 2011, Koretke *et al.*, 2000) and furthermore represent the two largest paralogous gene families in bacteria (Galperin, 2005). Since they are considerably less abundant in archaea and eukaryotes current state of research presumes multiple, independent lateral gene transfers of bacterial origin (Koretke et al., 2000). On average bacteria employ 25 TCSs to sense environmental factors (Barakat *et al.*, 2011), nevertheless there is a broad frequency range from 0 TCSs in *Mycoplasma genitalium*, 11 in *Helicobacter pylori*, 70 in *Bacillus subtilis*, up to 164 in *Streptomyces coelicolor* and 251 in *Myxococcus xanthus* (Heermann & Jung, 2010b). Census analyses of fully sequenced bacterial genomes suggest that the total number of two-component signaling genes grows as a square of genomic size (Galperin, 2005). Furthermore the number of two-component genes seems to strongly correlate with ecological and environmental niches (Alm *et al.*, 2006, Galperin *et al.*, 2001). Bacteria that live primarily in constant environments, like obligate intracellular parasites e.g. *Mycoplasma* or *Amoebophilus*, harbor only few signaling pathways, whereas a multitude of TCSs is employed by organisms dealing frequent fluctuations in their habitats (Capra & Laub, 2012). In the genome of *Escherichia coli* 30 sensory proteins, so called histidine kinases, and 32 effectors, so called response regulators are annotated. Many of these are part of intense studies, like the osmotic stress- and potassium sensing KdpD/KdpE systems (Heermann & Jung, 2010a) or the regulatory interplay of chemotaxis and aerotaxis around CheA/CheY (Thakor *et al.*, 2011). Figure 1.1 summarizes all TCSs of *E. coli* with their corresponding stimuli (if known) and cellular adaptations.

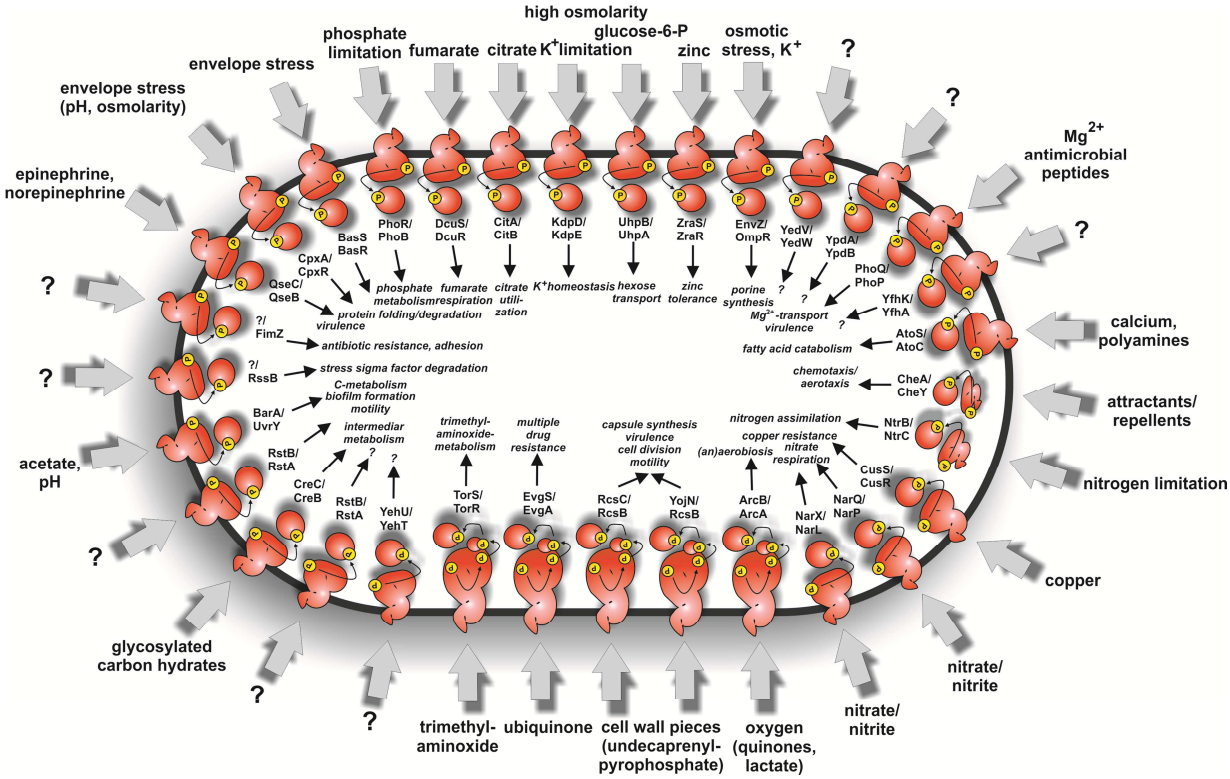

Figure 1.1 Two-component systems of *Escherichia coli* K-12. All histidine kinases (membrane-anchored, cytoplasmic or hybrid) and there cognate response regulator are summarized. Known stimuli and the corresponding cellular responses are indicated. Unknown components are depicted with (?). The figure was provided by Ralf Heermann, adapted and modified.

1.2 The paradigm of two-component signal transduction

The fundamental framework of a typical TCS includes a sensor histidine kinase (HK) and a cognate response regulator (RR) (Fig 1.2). Upon stimulus perception the histidine kinase typically catalyzes an ATP-dependent autophosphorylation reaction on a conserved histidine residue (Gao & Stock, 2009), resulting in a high-energy phosphoramidate. This process requires homodimerization of two histidine kinase proteins, whereas autophosphorylation itself can occur in two directions, *trans* (intersubunit) or *cis* (intrasubunit) within the dimeric histidine kinase (Casino *et al.*, 2014). In a second step this phosphoryl group of the HK is transferred to a conserved aspartate residue in a cognate RR protein (mixed acid anhydride). Usually phosphorylation of the RR results in a conformational change and causes an activation of the RR effector domain, which in turn modulates gene expression. This enables bacteria to coordinate their expression or physiology to changes of external or environmental conditions (Capra & Laub, 2012). Signal termination is mainly subject to two mechanisms: At first most HKs act bifunctional as phosphatases for their cognate response regulator in the absence of stimulating conditions (Kenney, 2010). Additionally the rapid turnover of phosphoanhydride bonds (half-life: s-min) is thought to further prevent excessive activation (Heermann & Jung, 2010b).

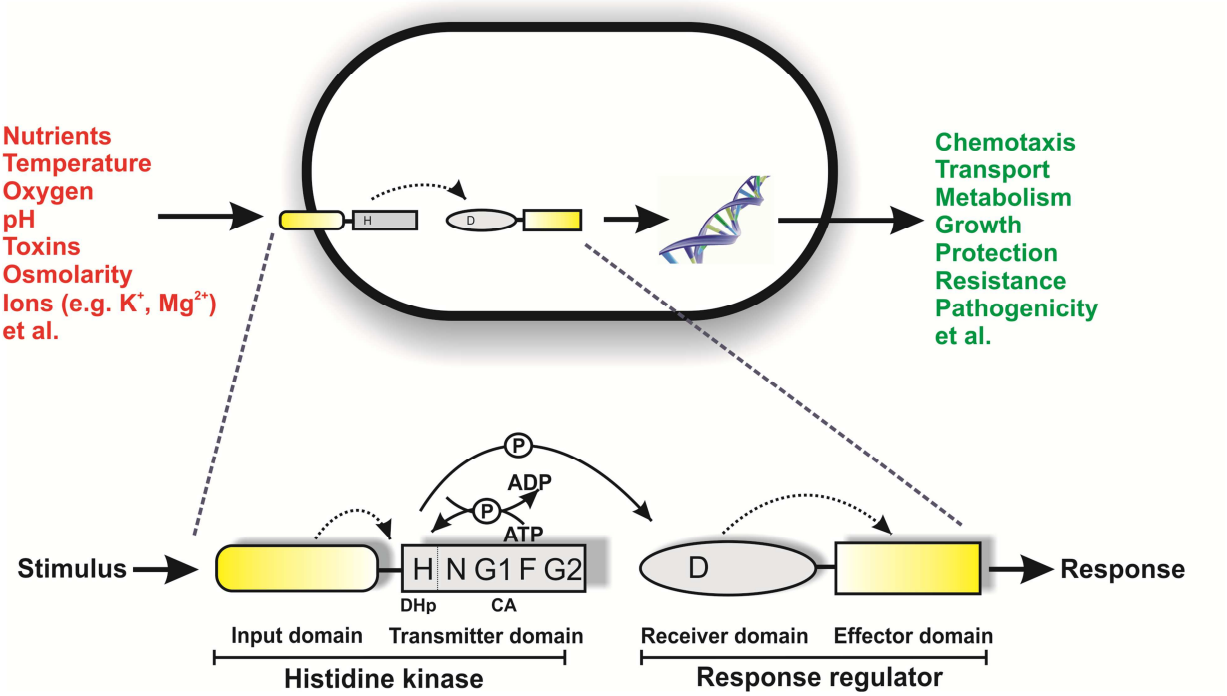

Figure 1.2 Signal transduction and domain assembly in the two-component system prototype. Histidine kinases (HKs) function as sensory elements for a variety of extra- and intracellular signals. Stimulus perception results in autophosphorylation of a conserved histidine residue. Transfer of this phosphoryl group to a conserved aspartate residue in the receiver domain a response regulator (RR) induces a conformational change, which in turn triggers cellular adaptions. The figure was provided by Ralf Heermann, adapted and modified.

The second most common variant of two-component signal transduction is the phosphorelay (Hoch, 2000). In this pathway the histidine kinase generally harbors an additional C-terminal receiver domain. Fifteen percent of all classified histidine kinases belong to this group of so called hybrid histidine kinases (Barakat et al., 2011). Upon autophosphorylation an intramolecular phosphotransfer occurs, before the phosphoryl group is shuttled via a histidine phosphotransferase (often referred to as histidine-containing phosphotransfer protein (HPt)) to its terminal response regulator. The extension of the signal transduction cascade facilitates different signal inputs/outputs, precise fine tuning and multiple cross connections (Hoch & Varughese, 2001) and is part of many complex processes, such as *Bacillus* sporulation (Scaramozzino *et al.*, 2009) or *Vibrio* quorum sensing based bioluminescence (Bassler *et al.*, 1997).

1.3 Structural and functional properties of histidine kinases

The centerpiece of all histidine kinases is the so called transmitter domain with two highly conserved subdomains: the dimerization and histidine phosphotransfer (DHp) domain (PFAM nomenclature: His Kinase A) including the highly conserved histidine phosphorylation site, and the catalytic and ATP-binding (CA) domain (PFAM nomenclature: HATPase_c), which harbors the catalytic activity of transferring the γ-phosphoryl-group of ATP to the histidine residue (Fig. 1.2) (Gao & Stock, 2009). Based on unique sequence motifs DHp- and CA domain can be further divided into the H-Box with the conserved histidine

residue (in DHp domain), and the N-, G1-, F- and G2-box (in the CA domain) essential for ATP binding (Stewart, 2010). Usually the majority of histidine kinases contain at least one, often more additional domains. The input domain, also referred to as signal recognition domain, is characterized by a large diversity and can harbor up to 13 transmembrane (TM-) domains (Galperin, 2005). Signal recognition predominantly occurs within the periplasmic or extracellular portion of the protein and modulates activity of the transmitter domain. Mechanistic insights into structural changes upon HK activation were obtained from different cytoplasmic structures e.g. from *E. coli* EnvZ (Tanaka *et al.*, 1998) or Streptococcus mutans VicK (Wang *et al.*, 2013). Based on the mechanism of signal perception and domain architecture, histidine kinases are classified into three subgroups: periplasmic- and extracellular sensing histidine kinases (e.g. EnvZ or VirA), membrane sensing histidine kinases (e.g. LytS or LuxN) and cytoplasmic sensing histidine kinases (e.g. KdpD or ArcB) (Mascher *et al.*, 2006). In addition, many histidine kinases contain further domains between the TM- and DHp- and CA domains. Most common members are PAS (from Per, Arnt and Sim proteins), HAMP (histidine kinases, adenylyl cyclases, methyl-accepting chemotaxis proteins and phosphatases) and GAF (cGMP specific phosphodiesterases, adenylyl cyclases and FhlA proteins) domains (Galperin et al., 2001). While HAMP domains often participate in signal transduction from input- to transmitter domain (e.g. in Tsr or Tar) (Parkinson, 2010), PAS- and GAF domains (e.g. in CitA) mainly function as additional signaling interfaces via ligand binding (Henry & Crosson, 2011, Möglich *et al.*, 2009, Unden *et al.*, 2013, Krell *et al.*, 2010). In general, sensor histidine kinases involved in asymmetric processes, like e.g. cell division, as it was observed for *Caulobacter crescentus* PleC (Viollier *et al.*, 2002), tend to exhibit an uneven, mostly polar subcellular distribution within the cell membrane. Although histidine kinases controlling metabolic processes do not show obvious localization requirements, DcuS and CitA, both carboxylate sensors in *E. coli*, were also found to accumulate at the cell poles (Scheu *et al.*, 2008, Kneuper *et al.*, 2010).

1.4 Structural and functional properties of response regulators

The vast majority of response regulators are characterized by two functionally separated domains, an N-terminal receiver domain and a C-terminal effector domain (Fig. 1.2). The most common receiver domain belongs to the structurally well conserved CheY-like type, which is characterized by a central five-stranded parallel β-sheet deriving from alternating α-helices and β-strands (Bourret, 2010). Within this structure several residues are highly conserved including the aspartate of the phosphorylation site. In close proximity to this site two further aspartates are crucial to recruit divalent cations, mainly Mg^{2+} (Lukat *et al.*, 1990), which is necessary to add or remove phosphoryl groups in the receiver domain. For some response regulators phosphorylation by small molecular weight molecules, like acetyl phosphate, has been described (Wolfe, 2010), nevertheless the physiological relevance remains elusive (Liu *et al.*, 2009). Upon phosphorylation conformational changes within the receiver domain mediate activation and subsequent dimerization of the response regulator (Stock *et al.*, 2000). Many effector domain protein families, which include enzymatic functions like methylestases (CheB-like), di-guanylate cyclases (GGDEF) or c-di-GMP-phosphodiesterases (EAL, HY-GYP) have been identified so far (see below). Nevertheless transcriptional regulation via DNA-binding represents the most common output response within this diversity (Galperin, 2006). Based on structural properties DNA-binding effector domains can be classified in different categories. With thirty percent OmpR/PhoB-like response regulator (PFAM nomenclature: Trans_reg_c) display the largest family and are characterized by winged helix domains. Recent studies on

KpdE, a well characterized member of this family, revealed detailed insights into asymmetric heterodomain processes, which are necessary to stabilize the response regulator-DNA complex (Narayanan *et al.*, 2014). The NarL/FixJ family is the second most abundant family of bacterial RRs. Its members possess a typical helix turn helix DNA-binding output domain (PFAM nomenclature: LuxR_c_like or GerE) with similarities to the transcriptional regulator LuxR (Galperin, 2010). Only three percent belong to the group of LytR/AgrA-like response regulator (PFAM nomenclature: LytTR). Recently the structure of the output domain of AgrA from *Staphylococcus aureus* was solved and revealed a novel mode of DNA-binding via three elongated β-sheets (Sidote *et al.*, 2008). Besides DNA-binding some effector domains direct alter enzymatic activity or mediate protein-protein interactions (Galperin, 2010). The response regulator VieA *controls* biofilm formation in *Vibrio cholera* (Tischler & Camilli, 2004), while its receiver domain is associated with an EAL domain and controls its c-di-GMP-specific phosphodiesterase activity (Tamayo *et al.*, 2005, Schmidt *et al.*, 2005). In contrast, mediation of protein-protein interactions upon receiver domain activation plays a key role in the chemotaxis machinery of *Bacillus subtilis* (Szurmant & Ordal, 2004). Here the presence of the N-terminal CheW-like domain in the response regulator CheV was shown to stabilize its phosphorylated state (Karatan *et al.*, 2001).

1.5 LytS/LytTR-like two-component systems

With very few exceptions LytS/LytTR-like two-component systems can be found in many bacterial genomes and represent the second-most distributed family of bacterial signal transduction genes (Geer *et al.*, 2002). The common element of all LytS-like histidine kinases is the signal recognition domain of the 5TM Lyt type (also referring to 5TMR-LYT or LytS-YhcK) (Anantharaman & Aravind, 2003). This domain with an average of 169 amino acids actually occurs in 88 different architectures and is predominantly found in sensor histidine kinases, but also appears in combination with GGDEF domains. Corresponding response regulators are characterized by an eponymous LytTR-like effector domain. This domain with an average of 96 amino acids can be further found in 25 different architectures, mainly in combination with a receiver domain (over 70 percent) or fused to four putative transmembrane domains (over 25 percent). LytS/LytTR-like two-component systems regulate several housekeeping or virulence genes in many bacterial pathogens (Table 1.1) (Galperin, 2008).

TABLE 1.1: LytS/LytTR two-component systems

TCS (HK/RR)	Organism	Regulated process	Reference
AgrC/AgrA	*Staphylococcus aureus*	Virulence, peptide quorum sensing	(Koenig *et al.*, 2004)
FimS/AlgR	*Pseudomonas aeruginosa*	Alginate production, type IV pilus function, virulence	(Lizewski *et al.*, 2004)
BlpH/BlpR	*Streptococcus pneumonia*	Bacteriocin production, peptide quorum sensing	(de Saizieu *et al.*, 2000)
BrsM/BrsR	*Streptococcus mutans*	Bacteriocin and mutacin production, peptide quorum sensing	(Xie *et al.*, 2010)
ComD/ComE	*Streptococcus pneumonia*	Competence, peptide quorum sensing	(Ween *et al.*, 1999)
FasB/FasA	*Streptococcus pyrogenes*	Fibronectin-binding adhesin production, streptolysin S	(Kreikemeyer *et al.*, 2001)
FsrC/FsrA	*Enterococcus faecalis*	Virulence, peptide quorum sensing	(Del Papa & Perego, 2011)
CabS/CabR PlnC/PlnD	*Lactobacillus planarum*	Bacteriocin production, peptide quorum sensing	(Risøen *et al.*, 2001)
LytS/LytR	*Staphylococcus aureus*	Peptidoglycan turnover, autolysis	(Brunskill & Bayles, 1996)
VirS/VirR	*Clostridium perfringens*	Production of exotoxins, collagenase, hemagglutinin	(Cheung & Rood, 2000)
HdrM/HdrR	*Streptococcus mutans*	Bacteriocin and lantibiotic mutacin production	(Okinaga *et al.*, 2010)
YehU/YehT	*Escherichia coli*	Carbon starvation control	(Chapter 2)
YpdA/YpdB	*Escherichia coli*	Carbon starvation control	(Chapter 3)

The LytS/LytR system from *S. aureus* controls bacterial autolysis and is hence associated with programmed cell death and peptidoglycan turnover during biofilm formation (Sadykov & Bayles, 2012). In *Pseudomonas aeruginosa*, an opportunistic human pathogen, the LytS/LytTR-like two-component system FimS/AlgR regulates biosynthesis of alginate, an extracellular polysaccharide (Lizewski et al., 2004). The two-component system ComD/ComE regulates natural competence in *Streptococcus pneumoniae*. Recently phosphorylation dependent dimerization of ComE was elucidated (Boudes *et al.*, 2014). One of the best characterized LytS/LytTR-like two-component systems is the AgrC/AgrA system *S. aureus*. The response regulator AgrA up-regulates genes encoding secreted virulence factors and down-regulates cell wall associated genes (Sidote et al., 2008). The group of Ann Stock succeeded solving the structural properties of the C-terminal effector domain of AgrA by x-ray crystallography in the presence of its target promoter DNA (Sidote et al., 2008). Two equally aligned AgrA proteins occupy their corresponding 9 bp recognition motifs, which are separated by a 12 bp spacer (Koenig et al., 2004). This interaction is mediated by three elongated β sheets (in almost parallel orientation) and revealed a hitherto unknown mode of DNA binding. Due to high diversity of LytTR-like effector domains an originally described LytTR DNA-binding consensus sequence (Nikolskaya & Galperin, 2002) might be much more variable as previously proposed (Del Papa & Perego, 2011). In addition, the Audette lab obtained first structural evidence for the receiver domain of LytR, a second LytTR-like response regulator in *S. aureus* (Shala *et al.*, 2013). As mentioned before receiver domains are conserved among different families of response regulators. Nevertheless, significant variability is an indispensable feature to limit cross talk and define specificity among two-component systems (Barbieri *et al.*, 2010, Podgornaia *et al.*, 2013).

1.5.1 The YehU/YehT two-component system of *Escherichia coli*

As described for many histidine kinase/response regulator-pairs (Williams & Whitworth, 2010), the genes encoding YehU/YehT are organized within a single operon (Fig. 1.3 A). This arrangement allows coordinated expression of both genes and is found to provide robustness within the signaling pathway (Løvdok *et al.*, 2009). The *yehUT* genes overlap for 4 bp and are located at 47.638 centisomes in *E. coli* MG1655 genome.

The LytS-like histidine kinase YehU consists of 561 amino acids (62.1 kDa) (Fig. 1.3 B). The signal recognition domain of YehU includes an N-terminal 5TM Lyt domain (PFAM nomenclature: 5TMR-LYT) and a GAF domain. Bioinformatic analyses for YehU predict six transmembrane helices with two outer and three inner loops [Data based on PSIPRED, TMHMM and OCTOPUS (Jones, 2007, Krogh *et al.*, 2001, Viklund & Elofsson, 2008)]. In general 5TM Lyt domains are characterized by an N/DxR motif between helix 1 and helix 2 and a multitude of small hydrophobic residues like glycine or proline, which might be involved in ligand binding or signal transmission (Anantharaman & Aravind, 2003). The additional GAF domain in cytoplasmic part of YehU is predicted by SMART, UniProt, and NCBI BlastP (Letunic *et al.*, 2006, Consortium, 2010, Johnson *et al.*, 2008). Although there are slide variations of the domain boundaries within the different programs, a GAF core motif can be defined from amino acid 218 to amino acid 365. GAF domains are present in a variety of different protein families and share structural similarities with the PAS domain family (Ho *et al.*, 2000). GAF domains are capable of binding small molecules (e.g. ions, cyclic nucleotides) and might affect signal transduction (Cann, 2007). Nevertheless

the question on their precise function in signaling compounds remains elusive (Möglich et al., 2009). The GAF domain in YehU is followed by a DHp domain and CA domain.

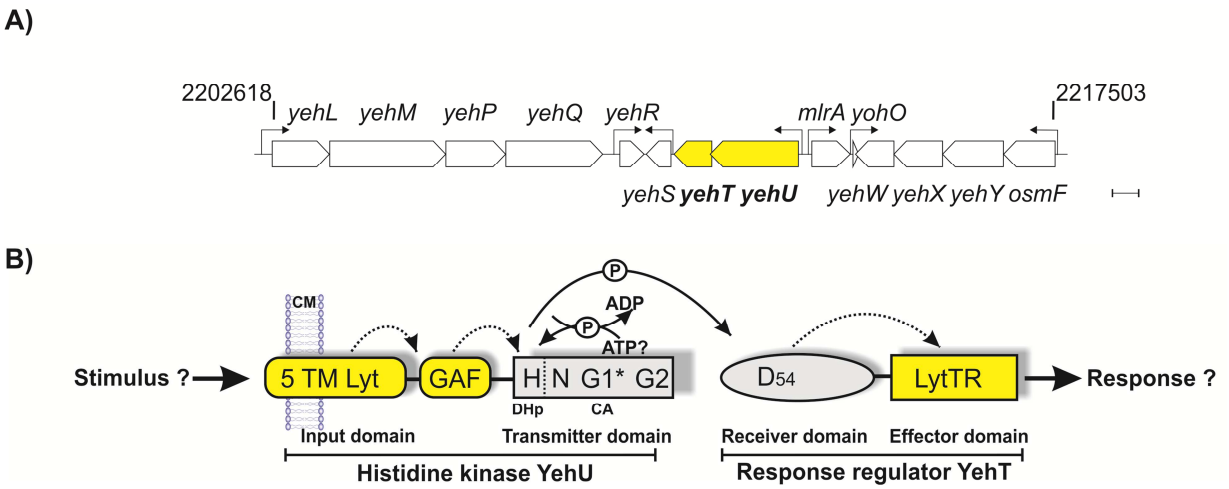

Figure 1.3 The YehU/YehT two-component system of *Escherichia coli*. A) The region between 47.48 and 47.77 centisomes (bp 2202618 to 2217503) depicts the chromosome around the *yehUT* locus of *E. coli* MG1655. Based on the database EcoCyc (http://www.ecocyc.org (Keseler *et al.*, 2009)) genes (boxes) and transcription start sites (arrows) are marked. The shown bar represents 500 bp. B) Predicted domain organization of YehU/YehT. The input domain of YehU is characterized by a membrane integrated 5TM Lyt domain and a GAF domain in the periplasm. The G1 box in the catalytically active transmitter domain is incomplete (hence G1*), binding of ATP as phosphor-donor for activation remains elusive (ATP?). YehT consists of a CheY-like receiver domain and LytTR-like DNA binding domain. Phosphorlyation sites are indicated (H, histidine 382 in YehU and D, aspartate 54 in YehT). CM, cytoplasmic membrane. The figure was provided by Luitpold Fried, adapted and modified.

The LytTR-like response regulator YehT consists of 239 amino acids (27.4 kDa) (Jain *et al.*, 2009) with an N-terminal CheY-like receiver domain and a C-terminal DNA-binding domain of the LytTR type (Fig. 1.3 B) (Finn *et al.*, 2010). Based on sequence analysis among CheY-like receiver domains (UniProt and NCBI BlastP) aspartate 54 is considered to be the phosphorylation site in YehT. Based on comparative secondary structure predictions and homology modeling to known LytTR-like effector domains [putative methyl-accepting/DNA response regulator from *Bacillus cereus* (RCS PBD ID: 3D6W) and AgrA from *S. aureus* (Sidote et al., 2008) (RCS PDB ID: 3BS1)] 99 percent of the YehT effector domain could be assigned (with a confidence of >90 percent), which suggests a similar elongated β-fold (Kelley & Sternberg, 2009).

In *E. coli* the physiological role of the YehU/YehT two-component system or its specific function is still unknown. Characterization of homologous two-component systems in Gram-positive organisms, e.g. *S. aureus* LytS/LytR (Sadykov & Bayles, 2012), revealed several candidate target genes, however none of these (if existent) were regulated in *E. coli* (Kraxenberger, 2011). Furthermore various comprehensive studies on YehU/YehT failed to identify either the target genes or the stimulus (Oshima *et al.*, 2002, Hirakawa *et al.*, 2003). Phenotypic analyses, testing over 2,000 different growth conditions (Zhou *et al.*, 2003, Lorenz, 2011), as well as studies focusing on cell motility, biofilm formation, cell surface hydrophobicity, curli formation or cell morphology could not identify any significant differences between

E. coli MG1655 and an isogenic *yehUT* mutant (Behr, 2009). In vitro characterization of all two-component systems revealed no viable evidence for YehU/YehT phosphorylation (Yamamoto *et al.*, 2005).

1.5.2 The YpdA/YpdB two-component system of *Escherichia coli*

The second LytS/LytTR-like two-component system in *E. coli* MG1655 comprises the LytS-like histidine kinase YpdA and the LytTR-like response regulator YpdB. The genes *ypdA* and *ypdB* form together with *ypdC* the *ypdABC* operon (53.56 centisomes) (Keseler et al., 2009).

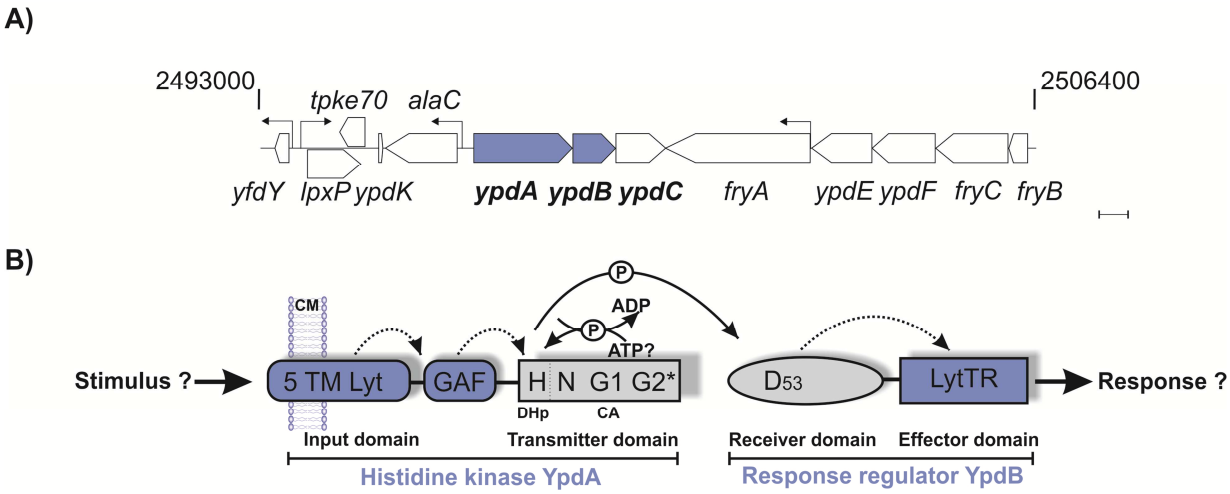

Figure 1.4 The YpdA/YpdB two-component system of *Escherichia coli*. A) The region between 53.73 and 54.01 centisomes (bp 2493000 to 2506400) depicts the chromosome around the *ypdABC* locus of *E. coli* MG1655. Based on the database EcoCyc (http://www.ecocyc.org (Keseler et al., 2009)) genes (boxes) and transcription start sites (arrows) are marked. The shown bar represents 500 bp. B) Predicted domain organization of YpdA and YpdB. The input domain of YpdA is characterized by a membrane integrated 5TM Lyt domain (Anantharaman & Aravind, 2003) and a GAF domain in the periplasm. The G2 box in the catalytically active transmitter domain is incomplete (hence G2*), binding of ATP as phosphor-donor for activation remains elusive (ATP?). YpdB consists of a CheY-like receiver domain and LytTR-like DNA binding domain. Phosphorylation sites are indicated (H, histidine 371 in YpdA and D, aspartate 53 in YpdB). CM, cytoplasmic membrane. The figure was provided by Luitpold Fried, adapted and modified.

The LytS-like histidine kinase YpdA consists of 565 amino acids (62.7 kDa). Its signal recognition domain includes an N-terminal 5TM Lyt domain with at least six transmembrane helices [Data based on PSIPRED, TMHMM and OCTOPUS (Jones, 2007, Krogh et al., 2001, Viklund & Elofsson, 2008)] and a GAF domain. As described for YehU the 5TM Lyt domain also displays the highly conserved N/DxR motif between helix 1 and helix 2 as well as a multitude of small hydrophobic residues like glycine or proline, which might be involved in ligand binding or signal transmission (Anantharaman & Aravind, 2003). The additional GAF domain might be capable of binding small molecules (e.g. ions, cyclic nucleotides) and hence affect signal transduction (Cann, 2007). Within the DHp domain of YpdA bioinformatics predict histidine 371 to be crucial for phosphorylation upon stimulus perception and activation of its adjacent CA domain (Letunic et al., 2006, Consortium, 2010, Johnson et al., 2008, Finn et al., 2010).

The LytTR-like response regulator YpdB consists of 244 amino acids (28.7 kDa) with an N-terminal CheY-like receiver domain and a C-terminal DNA-binding domain of the LytTR type. Based on sequence analysis among CheY-like receiver domains aspartate 53 is considered to be the phosphorylation site of YpdB. Results from comparative secondary structure predictions and homology modeling of YpdB (as described for YehT in 1.5.1) also suggest an elongated β fold within the DNA-binding domain (Kelley & Sternberg, 2009).

It is worth mentioning, that YpdA/YpdB and YehU/YehT share a high degree of similarity in *E. coli*: the histidine kinases have a sequence identity of 29 percent (sequence similarity of 53 percent), the response regulators of 32 percent (sequence similarity of 53 percent). Due to their high degree of sequence similarity correct phylogenetic assignment is challenging, but in contrast to YehU/YehT, the YpdA/YpdB two-component system seems to be less abundant, as it is missing in e.g. *Salmonella* species or *Yersinia* species (Franceschini *et al.*, 2013). Furthermore the transcriptional unit of the *ypdAB* operon is extended by an additional gene *ypdC*, which encodes a thus far undescribed AraC-like regulatory protein.

The physiological role and the specific function of the YpdA/YpdB two-component system are still unknown. Various comprehensive studies could not identify either the target genes or the stimulus of YpdA/YpdB (Oshima et al., 2002, Hirakawa et al., 2003). Phenotypic analyses, testing over 2,000 different growth conditions (Zhou et al., 2003, Lorenz, 2011), as well as studies focusing on a variety of growth-independent phenotypes could not identify any significant differences between *E. coli* MG1655 and an isogenic *ypdAB* mutant. Functional in vitro characterization of YpdA/YpdB remained elusive (Yamamoto et al., 2005). An adaptive evolutionary response towards accelerated growth on glucose has been reported to introduce a non-synonymous mutation in YpdA (S200A) (Aguilar *et al.*, 2012).

1.6 Scope of this thesis

LytS/LytTR-like two-component systems are well characterized in many Gram-positive bacteria. As mentioned before they have been intensively studied as they regulate a variety of virulence factors (e.g. toxin production) or virulence associated mechanisms (e.g. biofilm formation). In contrast only little is known about LytS/LytTR-like two-component systems in Gram-negative bacteria. The major aim of this study is to uncover the signal transduction mechanisms (signal perception and signal integration) of the LytS/LytTR-like YehU/YehT and YpdA/YpdB two-component systems in *Escherichia coli* in order to investigate their function and interconnectivity.

1.7 References for Introduction

Aguilar, C., A. Escalante, N. Flores, R. de Anda, F. Riveros-McKay, G. Gosset, E. Morett & F. Bolívar, (2012) Genetic changes during a laboratory adaptive evolution process that allowed fast growth in glucose to an *Escherichia coli* strain lacking the major glucose transport system. *BMC genomics* **13**: 385.

Alm, E., K. Huang & A. Arkin, (2006) The evolution of two-component systems in bacteria reveals different strategies for niche adaptation. *PLoS Comput. Biol.* **2**: e143.

Anantharaman, V. & L. Aravind, (2003) Application of comparative genomics in the identification and analysis of novel families of membrane-associated receptors in bacteria. *BMC Genomics* **4**: 34.

Barakat, M., P. Ortet & D. E. Whitworth, (2011) P2CS: a database of prokaryotic two-component systems. *Nucleic Acids Res.* **39**: D771-D776.

Barbieri, C. M., T. R. Mack, V. L. Robinson, M. T. Miller & A. M. Stock, (2010) Regulation of response regulator autophosphorylation through interdomain contacts. *J. Biol. Chem.* **285**: 32325-32335.

Bassler, B. L., E. P. Greenberg & A. M. Stevens, (1997) Cross-species induction of luminescence in the quorum-sensing bacterium *Vibrio harveyi*. *J. Bacteriol.* **179**: 4043-4045.

Behr, S., (2009) Expressionsanalyse YehU/YehT-regulierter Gene in *Escherichia coli*. *Diplomarbeit*: Ludwig-Maximilians-Universität München.

Boudes, M., D. Sanchez, M. Graille, H. van Tilbeurgh, D. Durand & S. Quevillon-Cheruel, (2014) Structural insights into the dimerization of the response regulator ComE from *Streptococcus pneumoniae*. *Nucleic Acids Res.*: gku110.

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

Brunskill, E. & K. Bayles, (1996) Identification and molecular characterization of a putative regulatory locus that affects autolysis in *Staphylococcus aureus*. *J. Bacteriol.* **178**: 611 - 618.

Cann, M., (2007) Sodium regulation of GAF domain function. *Biochem. Soc. Trans.* **35**: 1032-1034.

Capra, E. J. & M. T. Laub, (2012) Evolution of two-component signal transduction systems. *Annu. Rev. Microbiol.* **66**: 325-347.

Casino, P., L. Miguel-Romero & A. Marina, (2014) Visualizing autophosphorylation in histidine kinases. *Nat. Commun.* **5**.

Cheung, J. K. & J. I. Rood, (2000) The VirR response regulator from *Clostridium perfringens* binds independently to two imperfect direct repeats located upstream of the *pfoA* promoter. *J. Bacteriol.* **182**: 57.

Consortium, T. U., (2010) The Universal Protein Resource (UniProt) in 2010. *Nucleic Acids Res.* **38**: D142-D148.

de Saizieu, A., C. Gardes, N. Flint, C. Wagner, M. Kamber, T. J. Mitchell, W. Keck, K. E. Amrein & R. Lange, (2000) Microarray-based identification of a novel *Streptococcus pneumoniae* regulon controlled by an autoinduced peptide. *J. Bacteriol.* **182**: 4696-4703.

Del Papa, M. F. & M. Perego, (2011) *Enterococcus faecalis* virulence regulator FsrA binding to target promoters. *J. Bacteriol.* **193**: 1527.

Finn, R. D., J. Mistry, J. Tate, P. Coggill, A. Heger, J. E. Pollington, O. L. Gavin, P. Gunasekaran, G. Ceric, K. Forslund, L. Holm, E. L. L. Sonnhammer, S. R. Eddy & A. Bateman, (2010) The Pfam protein families database. *Nucleic Acids Res.* **38**: D211-D222.

Franceschini, A., D. Szklarczyk, S. Frankild, M. Kuhn, M. Simonovic, A. Roth, J. Lin, P. Minguez, P. Bork & C. von Mering, (2013) STRING v9. 1: protein-protein interaction networks, with increased coverage and integration. *Nucleic Acids Res.* **41**: D808-D815.

Galperin, M. Y., (2005) A census of membrane-bound and intracellular signal transduction proteins in bacteria: bacterial IQ, extroverts and introverts. *BMC Microbiol* **5**: 35.

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., (2008) Telling bacteria: do not LytTR. *Structure* **16**: 657-659.
Galperin, M. Y., (2010) Diversity of structure and function of response regulator output domains. *Curr. Opin. Microbiol.* **13**: 150-159.
Galperin, M. Y., A. N. Nikolskaya & E. V. Koonin, (2001) Novel domains of the prokaryotic two-component signal transduction systems. *FEMS Microbiol. Lett.* **203**: 11-21.
Gao, R. & A. M. Stock, (2009) Biological insights from structures of two-component proteins. *Annu. Rev. Microbiol.* **63**: 133-154.
Geer, L. Y., M. Domrachev, D. J. Lipman & S. H. Bryant, (2002) CDART: protein homology by domain architecture. *Genome Res.* **12**: 1619-1623.
Heermann, R. & K. Jung, (2010a) The complexity of the 'simple' two-component system KdpD/KdpE in *Escherichia coli*. *FEMS Microbiol. Lett.* **304**: 97-106.
Heermann, R. & K. Jung, (2010b) Stimulus perception and signaling in histidine kinases. In: Bacterial signaling. R. Krämer & K. Jung (eds). Wiley-VCH, Weinheim, pp. 135-161.
Henry, J. T. & S. Crosson, (2011) Ligand binding PAS domains in a genomic, cellular, and structural context. *Annu. Rev. Microbiol.* **65**: 261.
Hirakawa, H., K. Nishino, T. Hirata & A. Yamaguchi, (2003) Comprehensive studies of drug resistance mediated by overexpression of response regulators of two-component signal transduction systems in *Escherichia coli*. *J. Bacteriol.* **185**: 1851-1856.
Ho, Y.-S. J., L. M. Burden & J. H. Hurley, (2000) Structure of the GAF domain, a ubiquitous signaling motif and a new class of cyclic GMP receptor. *EMBO J* **19**: 5288-5299.
Hoch, J. A., (2000) Two-component and phosphorelay signal transduction. *Curr. Opin. Microbiol.* **3**: 165-170.
Hoch, J. A. & K. I. Varughese, (2001) Keeping signals straight in phosphorelay signal transduction. *J. Bacteriol.* **183**: 4941-4949.
Jain, E., A. Bairoch, S. Duvaud, I. Phan, N. Redaschi, B. Suzek, M. Martin, P. McGarvey & E. Gasteiger, (2009) Infrastructure for the life sciences: design and implementation of the UniProt website. *BMC Bioinformatics* **10**: 136.
Johnson, M., I. Zaretskaya, Y. Raytselis, Y. Merezhuk, S. McGinnis & T. L. Madden, (2008) NCBI BLAST: a better web interface. *Nucleic Acids Res.* **36**: W5-9.
Jones, D. T., (2007) Improving the accuracy of transmembrane protein topology prediction using evolutionary information. *Bioinformatics* **23**: 538-544.
Karatan, E., M. M. Saulmon, M. W. Bunn & G. W. Ordal, (2001) Phosphorylation of the Response Regulator CheV Is Required for Adaptation to Attractants during *Bacillus subtilis* Chemotaxis. *J. Biol. Chem.* **276**: 43618-43626.
Kelley, L. A. & M. J. Sternberg, (2009) Protein structure prediction on the Web: a case study using the Phyre server. *Nature Protoc.* **4**: 363-371.
Kenney, L. J., (2010) How important is the phosphatase activity of sensor kinases? *Curr. Opin. Microbiol.* **13**: 168-176.
Keseler, I. M., C. Bonavides-Martínez, J. Collado-Vides, S. Gama-Castro, R. P. Gunsalus, D. A. Johnson, M. Krummenacker, L. M. Nolan, S. Paley, I. T. Paulsen, M. Peralta-Gil, A. Santos-Zavaleta, A. G. Shearer & P. D. Karp, (2009) EcoCyc: A comprehensive view of *Escherichia coli* biology. *Nucleic Acids Res.* **37**: D464-D470.
Kneuper, H., P. D. Scheu, M. Etzkorn, M. Sevvana, P. Dünnwald, S. Becker, M. Baldus, C. Griesinger & G. Unden, (2010) Sensing ligands by perplasmic sensing histidine kinases with sensory PAS domains. In: Sensory mechanisms in bacteria S. Spiro & R. Dixon (eds). Caister Academic Press, Norfolk UK, pp. 39-60.

Koenig, R. L., J. L. Ray, S. J. Maleki, M. S. Smeltzer & B. K. Hurlburt, (2004) *Staphylococcus aureus* AgrA binding to the RNAIII-agr regulatory region. *J. Bacteriol.* **186**: 7549-7555.

Koretke, K. K., A. N. Lupas, P. V. Warren, M. Rosenberg & J. R. Brown, (2000) Evolution of two-component signal transduction. *Mol. Biol. Evol.* **17**: 1956-1970.

Kraxenberger, T., (2011) Zur Funktion des Sensor-Histidinkinase/Antwortregulator Systems YehU/YehT in *Escherichia coli*. *Dissertation*: Ludwig-Maximilians-Universität München.

Kreikemeyer, B., M. D. Boyle, B. A. L. Buttaro, M. Heinemann & A. Podbielski, (2001) Group A streptococcal growth phase-associated virulence factor regulation by a novel operon (*fas*) with homologies to two-component type regulators requires a small RNA molecule. *Mol. micro.* **39**: 392-406.

Krell, T., J. Lacal, A. Busch, H. Silva-Jiménez, M.-E. Guazzaroni & J. L. Ramos, (2010) Bacterial sensor kinases: Diversity in the recognition of environmental signals. *Annu. Rev. Microbiol.* **64**: 539-559.

Krogh, A., B. Larsson, G. von Heijne & E. Sonnhammer, (2001) Predicting transmembrane protein topology with a hidden Markov model: application to complete genomes. *J. Mol. Evol.* **305**: 567 - 580.

Letunic, I., R. R. Copley, B. Pils, S. Pinkert, J. Schultz & P. Bork, (2006) SMART 5: domains in the context of genomes and networks. *Nucleic Acids Res.* **34**: D257-260.

Liu, X., G. R. Pena Sandoval, B. L. Wanner, W. S. Jung, D. Georgellis & O. Kwon, (2009) Evidence against the physiological role of acetyl phosphate in the phosphorylation of the ArcA response regulator in *Escherichia coli*. *J. Microbiol.* **47**: 657-662.

Lizewski, S. E., J. R. Schurr, D. W. Jackson, A. Frisk, A. J. Carterson & M. J. Schurr, (2004) Identification of AlgR-regulated genes in *Pseudomonas aeruginosa* by use of microarray analysis. *J. Bacteriol.* **186**: 5672-5684.

Lorenz, N., (2011) Der Einfluss von Zwei-Komponenten-Systemen mit LytTR-artigen Antwortregulatoren auf den Phänotyp und die Expression potentieller Zielgene in *Escherichia coli*. *Diplomarbeit*: Ludwig-Maximilians-Universität München.

Løvdok, L., K. Bentele, N. Vladimirov, A. Müller, F. S. Pop, D. Lebiedz, M. Kollmann & V. Sourjik, (2009) Role of translational coupling in robustness of bacterial chemotaxis pathway. *PLoS Biol.* **7**: e1000171.

Lukat, G. S., A. M. Stock & J. B. Stock, (1990) Divalent metal ion binding to the CheY protein and its significance to phosphotransfer in bacterial chemotaxis. *Biochemistry* **29**: 5436-5442.

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

Möglich, A., R. A. Ayers & K. Moffat, (2009) Structure and signaling mechanism of Per-ARNT-Sim domains. *Structure* **17**: 1282-1294.

Narayanan, A., S. Kumar, A. N. Evrard, L. N. Paul & D. A. Yernool, (2014) An asymmetric heterodomain interface stabilizes a response regulator–DNA complex. *Nat. Comm.* **5**.

Nikolskaya, A. N. & M. Y. Galperin, (2002) A novel type of conserved DNA-binding domain in the transcriptional regulators of the AlgR/AgrA/LytR family. *Nucleic Acids Res.* **30**: 2453-2459.

Okinaga, T., G. Niu, Z. Xie, F. Qi & J. Merritt, (2010) The *hdrRM* operon of *Streptococcus mutans* encodes a novel regulatory system for coordinated competence development and bacteriocin production. *J. Bacteriol.* **192**: 1844-1852.

Oshima, T., H. Aiba, Y. Masuda, S. Kanaya, M. Sugiura, B. L. Wanner, H. Mori & T. Mizuno, (2002) Transcriptome analysis of all two-component regulatory system mutants of *Escherichia coli* K-12. *Mol. Microbiol.* **46**: 281-291.

Parkinson, J. S., (2010) Signaling mechanisms of HAMP domains in chemoreceptors and sensor Kinases. *Annu. Rev. Microbiol.* **64**: 101-122.

Podgornaia, A. I., P. Casino, A. Marina & M. T. Laub, (2013) Structural basis of a rationally Rewired Protein-protein interface critical to bacterial Signaling. *Structure* **21**: 1636-1647.

Risøen, P., O. Johnsborg, D. Diep, L. Hamoen, G. Venema & I. Nes, (2001) Regulation of bacteriocin production in *Lactobacillus plantarum* depends on a conserved promoter arrangement with consensus binding sequence. *Mol. Genet. Genomics* **265**: 198-206.

Sadykov, M. R. & K. W. Bayles, (2012) The control of death and lysis in staphylococcal biofilms: a coordination of physiological signals. *Curr. Opin. Microbiol.* **15**: 211-215.

Scaramozzino, F., A. White, M. Perego & J. A. Hoch, (2009) A unique GTP-dependent sporulation sensor histidine kinase in *Bacillus anthracis*. *J Bacteriol* **191**: 687-692.

Schaller, G. E., S.-H. Shiu & J. P. Armitage, (2011) Two-component systems and their co-option for eukaryotic signal transduction. *Curr. Biol.* **21**: R320-R330.

Scheu, P., S. Sdorra, Y.-F. Liao, M. Wegner, T. Basché, G. Unden & W. Erker, (2008) Polar accumulation of the metabolic sensory histidine kinases DcuS and CitA in *Escherichia coli*. *Microbiology* **154**: 2463-2472.

Schmidt, A. J., D. A. Ryjenkov & M. Gomelsky, (2005) The ubiquitous protein domain EAL is a cyclic diguanylate-specific phosphodiesterase: enzymatically active and inactive EAL domains. *J. bacteriol.* **187**: 4774-4781.

Shala, A., K. H. Patel, D. Golemi-Kotra & G. F. Audette, (2013) Expression, purification, crystallization and preliminary X-ray analysis of the receiver domain of *Staphylococcus aureus* LytR protein. *Acta. Crystallogr. Sect. F Struct. Biol. Cryst. Commun.* **69**: 1418-1421.

Sidote, D. J., C. M. Barbieri, T. Wu & A. M. Stock, (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.

Stewart, R. C., (2010) Protein histidine kinases: assembly of active sites and their regulation in signaling pathways. *Curr. Opin. Microbiol.* **13**: 133-141.

Stock, A. M., V. L. Robinson & P. N. Goudreau, (2000) Two-component signal transduction. *Ann. Rev. Biochem.* **69**: 183-215.

Szurmant, H. & G. W. Ordal, (2004) Diversity in chemotaxis mechanisms among the bacteria and archaea. *Microbiol. Mol. Biol. R.* **68**: 301-319.

Tamayo, R., A. D. Tischler & A. Camilli, (2005) The EAL domain protein VieA is a cyclic diguanylate phosphodiesterase. *J. Biol. Chem.* **280**: 33324-33330.

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

Thakor, H., S. Nicholas, I. M. Porter, N. Hand & R. C. Stewart, (2011) Identification of an anchor residue for CheA-CheY interactions in the chemotaxis system of *Escherichia coli*. *J. Bacteriol.* **193**: 3894-3903.

Tischler, A. D. & A. Camilli, (2004) Cyclic diguanylate (c-di-GMP) regulates *Vibrio cholerae* biofilm formation. *Mol. Microbiol.* **53**: 857-869.

Unden, G., S. Nilkens & M. Singenstreu, (2013) Bacterial sensor kinases using Fe–S cluster binding PAS or GAF domains for O2 sensing. *Dalton Trans.* **42**: 3082-3087.

Viklund, H. & A. Elofsson, (2008) OCTOPUS: improving topology prediction by two-track ANN-based preference scores and an extended topological grammar. *Bioinformatics* **24**: 1662-1668.

Viollier, P. H., N. Sternheim & L. Shapiro, (2002) A dynamically localized histidine kinase controls the asymmetric distribution of polar pili proteins. *EMBO J.* **21**: 4420-4428.

Wang, C., J. Sang, J. Wang, M. Su, J. S. Downey, Q. Wu, S. Wang, Y. Cai, X. Xu & J. Wu, (2013) Mechanistic insights revealed by the crystal structure of a histidine kinase with signal transducer and sensor domains. *PLoS Biol.* **11**: e1001493.

Ween, O., P. Gaustad & L. S. Havarstein, (1999) Identification of DNA binding sites for ComE, a key regulator of natural competence in *Streptococcus pneumoniae*. *Mol. Microbiol.* **33**: 817-827.

Williams, R. H. & D. E. Whitworth, (2010) The genetic organisation of prokaryotic two-component system signalling pathways. *BMC Genomics* **11**: 720.

Wolfe, A. J., (2010) Physiologically relevant small phosphodonors link metabolism to signal transduction. *Curr. Opin. Microbiol.* **13**: 204-209.

Xie, Z., T. Okinaga, G. Niu, F. Qi & J. Merritt, (2010) Identification of a novel bacteriocin regulatory system in *Streptococcus mutans*. *Mol. Microbiol.* **78**: 1431-1447.

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

Zhou, L., X.-H. Lei, B. R. Bochner & B. L. Wanner, (2003) Phenotype microarray analysis of *Escherichia coli* K-12 mutants with deletions of all two-component systems. *J. Bacteriol.* **185**: 4956-4972.

2 First Insights into the Unexplored Two-Component System YehU/YehT in *Escherichia coli*

Tobias Kraxenberger#, Luitpold Fried#, Stefan Behr and Kirsten Jung*

Munich Center for Integrated Protein Science (CIPSM) at the Department of Microbiology, Ludwig-Maximilians-Universität München, 82152 Martinsried, Germany

# These authors contributed equally to this work

*To whom correspondence should be addressed:
Dr. Kirsten Jung
Ludwig-Maximilians-Universität München
Department Biologie I, Bereich Mikrobiologie
Großhaderner Str. 2-4
82152 Martinsried
Germany
Phone: +49-89-2180-74500
Fax: +49-89-2180-74520
E-mail: jung@lmu.de

Abstract

Two-component systems (TCSs) consisting of a membrane-anchored histidine kinase (HK) and a response regulator (RR) are major players in signal transduction in prokaryotes. Whereas most TCSs in *Escherichia coli* are well characterized, almost nothing is known about the LytS-like HK YehU and the corresponding LytTR-like RR YehT. To identify YehT-regulated genes, we compared the transcriptomes of *E. coli* cells overproducing either YehT or the RR KdpE (control). The expression levels of 32 genes varied by more than 8-fold between the two strains. A comprehensive evaluation of these genes identified *yjiY* as a target of YehT. Electrophoretic mobility shift assays with purified YehT confirmed that YehT interacts directly with the *yjiY* promoter. Specifically, YehT binds to two direct repeats of the motif ACC[G/A]CT[C/T]A separated by a 13-bp spacer in the *yjiY* promoter. The target gene *yjiY* encodes an inner membrane protein belonging to the CstA superfamily of transporters. In *E. coli* cells growing in media containing peptides or amino acids as carbon source, *yjiY* is strongly induced at the onset of the stationary growth phase. Moreover, expression was found to be dependent on cAMP/CRP. It is suggested that YehU/YehT participates in the stationary phase control network.

Full-text article:

http://www.ncbi.nlm.nih.gov/pubmed/22685278
http://jb.asm.org/content/194/16/4272.long

Journal of Bacteriology, (2012), T. Kraxenberger, L. Fried, S. Behr and K. Jung, First Insights into the Unexplored Two-Component System YehU/YehT in *Escherichia coli*. Vol. 194, No. 16, p. 4272-4284, doi: 10.1128/JB.00409-12, American Society for Microbiology, Copyright © 2012, American Society for Microbiology.

3 Identification of a Target Gene and Activating Stimulus for the YpdA/YpdB Histidine Kinase/Response Regulator System in *Escherichia coli*

Luitpold Fried#, Stefan Behr#, and Kirsten Jung*

Munich Center for Integrated Protein Science (CIPSM) at the Department of Microbiology, Ludwig-Maximilians-Universität München, 82152 Martinsried, Germany

# These authors contributed equally to this work

*To whom correspondence should be addressed:
Dr. Kirsten Jung
Ludwig-Maximilians-Universität München
Department Biologie I, Bereich Mikrobiologie
Großhaderner Str. 2-4
82152 Martinsried
Germany
Phone: +49-89-2180-74500
Fax: +49-89-2180-74520
E-mail: jung@lmu.de

Abstract

Escherichia coli contains 30 two-component systems (TCSs), each consisting of a histidine kinase and a response regulator. Whereas most TCSs are well characterized in this model organism, little is known about the YpdA/YpdB system. To identify YpdB-regulated genes, we compared the transcriptomes of E. coli cells overproducing either YpdB or a control protein. Expression levels of 15 genes differed by more than 1.9-fold between the two strains. A comprehensive evaluation of these genes identified *yhjX* as the sole target of YpdB. Electrophoretic mobility shift assays with purified YpdB confirmed its interaction with the *yhjX* promoter. Specifically, YpdB binds to two direct repeats of the motif GGCATTTCAT separated by an 11-bp spacer in the yhjX promoter. *yhjX* encodes a cytoplasmic membrane protein of unknown function that belongs to the major facilitator superfamily of transporters. Finally, we characterized the pattern of *yhjX* expression and identified extracellular pyruvate as a stimulus for the YpdA/YpdB system. It is suggested that YpdA/YpdB contributes to nutrient scavenging before entry into stationary phase.

Full-text article:

http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3562098/

http://jb.asm.org/content/195/4/807.long

Journal of Bacteriology, (2013), L. Fried, S. Behr and K. Jung, Identification of a Target Gene and Activating Stimulus for the YpdA/YpdB Histidine Kinase/Response Regulator System in *Escherichia coli*. Vol. 195, No. 4, p. 807-815, doi: 10.1128/JB.02051-12, American Society for Microbiology, Copyright © 2013, American Society for Microbiology.

4 Identification of a Novel Nutrient-Sensing Histidine Kinase/Response Regulator Network in *Escherichia coli*

Stefan Behr#, Luitpold Fried#, and Kirsten Jung*

Munich Center for Integrated Protein Science (CIPSM) at the Department of Microbiology, Ludwig-Maximilians-Universität München, 82152 Martinsried, Germany

# These authors contributed equally to this work

*To whom correspondence should be addressed:
Dr. Kirsten Jung
Ludwig-Maximilians-Universität München
Department Biologie I, Bereich Mikrobiologie
Großhaderner Str. 2-4
82152 Martinsried
Germany
Phone: +49-89-2180-74500
Fax: +49-89-2180-74520
E-mail: jung@lmu.de

Abstract

When carbon sources become limiting for growth, bacteria must choose which of the remaining nutrients should be used first. We have identified a nutrient-sensing signaling network in *Escherichia coli* that is activated at the transition to stationary phase. The network is composed of the two histidine kinase/response regulator systems YehU/YehT and YpdA/YpdB and their target proteins, YjiY and YhjX (both of which are membrane-integrated transporters). The peptide/amino acid-responsive YehU/YehT system was found to have a negative effect on expression of the target gene, *yhjX*, of the pyruvate-responsive YpdA/YpdB system, while the YpdA/YpdB system stimulated expression of *yjiY*, the target of the YehU/YehT system. These effects were confirmed in mutants lacking any of the genes for the three primary components of either system. Furthermore, an in vivo interaction assay based on bacterial adenylate cyclase detected heteromeric interactions between the membrane-bound components of the two systems, specifically, between the two histidine kinases and the two transporters, which is compatible with the formation of a larger signaling unit. Finally, the carbon storage regulator A (CsrA) was shown to be involved in posttranscriptional regulation of both *yjiY* and *yhjX*.

Full-text article:

http://jb.asm.org/content/196/11/2023.long

Journal of Bacteriology, (2014), S. Behr, L. Fried and K. Jung, Identification of a novel nutrient sensing histidine kinase/response regulator network in *Escherichia coli*. Vol.196 , No.11 , p. 2023-2029, doi: 10.1128/JB.01554-14, American Society for Microbiology, Copyright © 2014, American Society for Microbiology.

5 Characterization of the accessory protein YehS in LytS/LytTR mediated signal transduction of *Escherichia coli*

5.1 Introduction

Recently more and more proteins have been identified, which enrich the classical image of two-component signal transduction by integration of additional functions into the main signal-response mechanism. These so called accessory proteins were considered exceptions, but the increasing number of reports on three- (or more) component systems indicates that such systems are more frequent than initially anticipated (Krell *et al.*, 2010). Accessory proteins can be found in all bacterial compartments and possess a broad range of functional properties, like signal integration or scaffolding, and interfere at various signal transduction sites (Jung *et al.*, 2012). The major proportion of these proteins acts on histidine kinases influencing signal perception and transduction (Buelow & Raivio, 2010). Hence the co-sensing function of transport proteins, which has intensively studied for e.g. the lysine permease LysP and the pH-sensor CadC in *E. coli* (Tetsch *et al.*, 2008, Rauschmeier *et al.*, 2014), is a common mechanism to integrate information on metabolite fluxes into transcriptional regulation. Scaffolding proteins often coordinate the physical assembly of signaling compounds. The universal stress protein UspC e.g. acts as scaffold for the KdpD/KdpE signaling cascade in *E. coli*. Under conditions of high osmolarity (salt stress) an interaction of UspC with the histidine kinase KdpD promotes the expression of the *kdpFABC* operon encoding a high-affinity K^+ uptake system (Heermann *et al.*, 2009). Connector proteins in general mediate and/or coordinate the output response of two individual signaling cascades. In *Salmonella enterica* e.g. the TCSs PhoP/PhoQ (responding to low extracellular Mg^{2+} concentrations (Shin *et al.*, 2006)) and PmrA/PmrB (responding to Fe^{3+}) are coordinated via the connector protein PmrD to mediate resistence against polypeptide antibiotics (Kox *et al.*, 2000).

We identified with *yehS* in the genomic neighborhood of the *yehUT* operon (Fig. 5.1 A) a gene encoding for a potential accessory protein. YehS consists of 156 amino acids (approximately 18.0 kDa) and harbors a duplication (from amino acids 3 to 78 and amino acids 86 to 153) of an uncharacterized domain (Pfam nomenclature: DUF 1456) (Fig. 5.1 B). Although this protein lacks any evidence so far, its architectural domain assembly (Finn *et al.*, 2010) seems to be of functional importance. Its distribution is predominantly limited to γ-proteobacteria where it is always associated with (LytS/LytTR-) signaling genes. On the other site it is not essential as homologs are missing in e.g. *Photorhapdus luminescens* or *Vibrio harveyi* (Franceschini *et al.*, 2013).

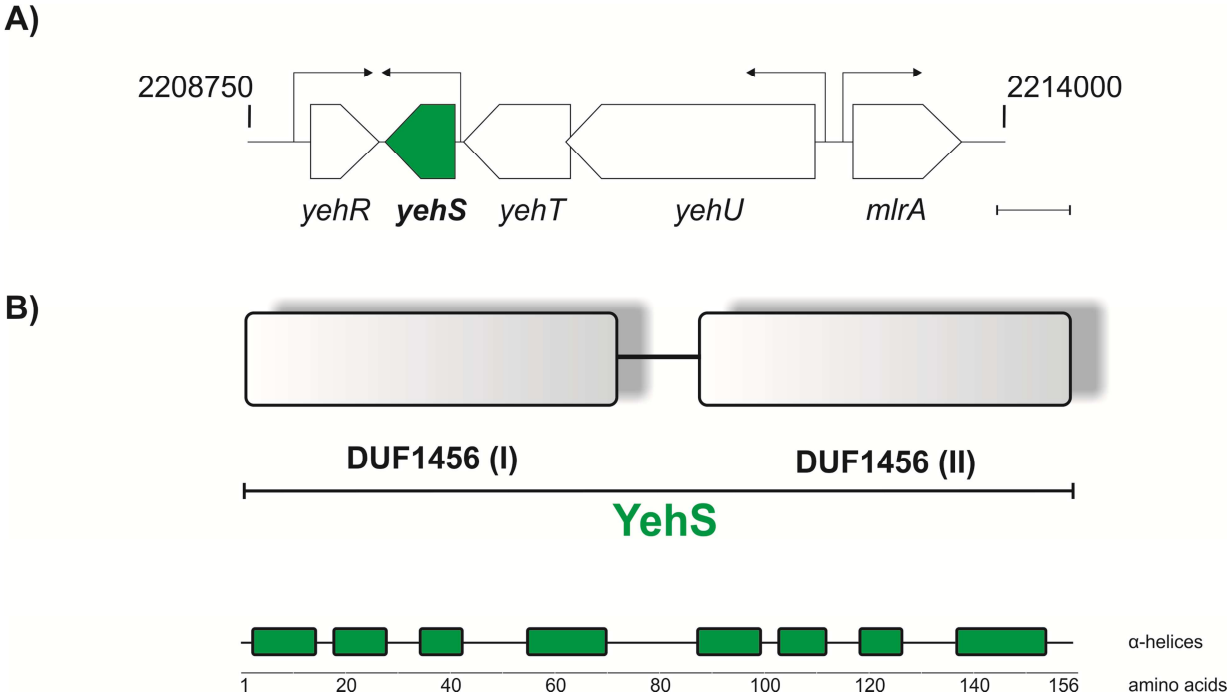

Fig. 5.1 The accessory protein YehS. A) The region around the genomic locus of *yehS* in *E. coli* MG1655 is shown between centisomes 47.6 and 47.7 (bp 2208750 and 22014000). Based on the scientific database EcoCyc (http://www.ecocyc.org, (Keseler *et al.*, 2009)) genes (boxes) and transcription start sites (arrows) are marked. The shown bar represents 500 bp. B) Predicted domain organization of YehS suggests a duplication of a hitherto unknown domain called DUF1456. Several structural prediction analyses (PSIPRED, JPRED3, PROFsec) indicate four α-helical elements in both subdomains.

Primary structure analysis revealed a theoretical isoelectric point of 9.2. Secondary structure predictions with PSIPRED, JPRED3 or PROFsec suggest four α-helical elements in each domain (McGuffin *et al.*, 2000, Cole *et al.*, 2008). Our investigations focused on the function of YehS in the LytS/LytTR mediated signal transduction network of *E. coli* to gain further insights into its complex regulatory interplay.

5.2 Material and Methods

Strains, plasmids and oligonucleotides. *E. coli* strains and their genotypes are listed in Table 5.1. Mutants were constructed by using the *E. coli* Quick-and-Easy Gene Deletion Kit (Gene Bridges) and the Bac Modification Kit (Gene Bridges) as reported (Heermann *et al.*, 2008). Both kits rely on the Red®/ET® recombination technique. Plasmids and oligonucleotide are listed in Table 5.1. DNA fragments for plasmid construction were amplified by PCR from genomic DNA.

Molecular biological techniques. Plasmid DNA and genomic DNA were isolated using the HiYield Plasmid Mini-Kit (Suedlaborbedarf) and the DNeasy Blood and Tissue Kit (Qiagen), respectively. DNA fragments

were purified from agarose gels using the HiYield PCR Clean-up & Gel Extraction Kit (Suedlaborbedarf). Q5 DNA polymerase (New England Biolabs) was used according to the supplier's instructions. Restriction enzymes and other DNA-modifying enzymes were also purchased from New England Biolabs and used according to the manufacturer's directions.

In vivo protein-protein interaction studies using BACTH. Protein-protein interactions were assayed with the bacterial adenylate cyclase-based two-hybrid system (BACTH) essentially as described previously (Karimova, 2005, Behr *et al.*, 2014). *E. coli* BTH101 was transformed with different pUT18, pUT18C and pKT25, pKNT25 derivatives (Table 5.1) to test for interactions. Cells were grown under aeration overnight in LB medium supplemented with 0.5 mM isopropyl-β-D-thiogalactopyranoside (IPTG) at 30°C and harvested for determination of β-galactosidase activities, which are expressed in Miller units (Miller, 1992).

In vivo expression studies. In vivo expression of *yhjX* and *yjiY* was quantified by means of luciferase-based reporter gene assays, using *E. coli* MG1655 cells that had been transformed with the plasmids pBBR *yjiY-lux* or pBBR *yhjX-lux,* respectively (Table 5.1).

Cells of an overnight culture grown in M9 minimal medium with 0.4% (w/v) glucose as C-source were inoculated into M9 minimal medium [supplemented with 20 mM pyruvate (for pBBR *yhjX-lux*) or 0.4% casamino acids (for pBBR *yjiY-lux*)] to give a starting OD_{600} of 0.05. Cells were then incubated under aerobic growth conditions at 37°C, and OD_{600} and luminescence were measured continuously. Optical density was determined in a microplate reader (Tecan Sunrise) at 600 nm. Luminescence levels were determined in a luminescence reader (Centro LB960, Berthold Technology) for 0.1 s, and are reported as relative light units [counts s^{-1}] (RLU).

Overproduction and Purification of 6His-YehS. *E. coli* BL21(DE3) was transformed with pBAD24-his *yehS*. After inoculation in LB medium (OD_{600}=0.05) cells were grown aerobically at 37°C to an OD_{600} of 0.5 before overproduction of 6His-YehS was induced by the addition of 0.4% (w/v) L-arabinose. Cells were harvested after 3 hours of induction. Purification of 6His-YehS was performed as described before (Kraxenberger *et al.*, 2012) using 50 mM Tris/HCl pH 7.5, 5% (v/v) glycerol with 2 mM dithiothreitol as standard purification buffer. Protein concentration was determined by the method of Lowry (Lowry *et al.*, 1951) or photometric (PeqLab, Nano-Drop ND-1000) using predictions for YehS extinction coefficient (Abs 0.1% (=1 g/l) = 0.555) from ProtParam (http://www.expasy.ch/tools/protparam.html). Production, fractionation and purification was follow by SDS-PAGE (Laemmli, 1970) and Western Blot with primary anti-His antibody (Qiagen).

Analytical size-exclusion chromatography (SEC) and Right Angle Light Scattering (RALS). After purification 6His-YehS was analyzed via SEC/RALS using a *Superdex S200 increase 5/150GL* column on an ÄKTAmicro system. The run was performed with a flow rate of 0.5 ml/min in sample buffer (50 mM Tris/HCl pH 7.5, 5% (v/v) glycerol, 2 mM dithiothreitol) and A_{280} was monitored. A BioRad gel filtration standard was used as a reference. Consecutive right angle light scattering (RALS) was performed on a Viscotek RI detector. All experiments were performed in cooperation with Dr. Gregor Witte from the AG Hopfner group in the Gene Center and Department of Biochemistry of the Ludwig-Maximilians-University, München.

TABLE 5.1. Bacterial strains and plasmids used in this study

Name	Relevant genotype or description	Reference
***E. coli* strains**		
MG1655	$F^- \lambda^-$ *ilvG rfb50 rph-1*	(Blattner *et al.*, 1997)
ΔyehS	MG1655 *rpsL150 yehS*::*rpsL-neo*; Kan^r Str^s	This work
BL21(DE3)	F^- *ompT hsdSB*(r^-_B, m^-_B) *gal dcm* (DE3)	(Studier & Moffatt, 1986)
BTH101	F^- *cyaA*-99 *araD*139 *galE*15 *galK*16 *rpsL*1 *hsdR*2 µ*rA1* µ*rB1*	(Karimova *et al.*, 1998)
DH5α	F^- *endA*1 *glnV*44 *thi*-1 *recA*1 *relA*1 *gyrA*96 *deoR nupG* Φ80d*lacZ*ΔM15 Δ(*lacZYA-argF*)U169, *hsdR*17(r_K^- m_K^+), λ^-	(Meselson & Yuan, 1968)
Plasmids		
pBBR *yjiY-lux*	P_{yjiY}-212/+88 cloned in the BamHI and EcoRI sites of pBBR1-MCS5-TT-RBS-lux; Gm^r	(Kraxenberger *et al.*, 2012)
pBBR *yhjX-lux*	P_{yhjX}-264/+36 cloned in the BamHI and EcoRI sites of pBBR1-MCS5-TT-RBS-lux; Gm^r	(Fried *et al.*, 2013)
pBAD24	Arabinose-inducible PBAD promoter, pBR322 ori; Amp^r	(Guzman *et al.*, 1995)
pBAD24-his *yehS*	*yehS* cloned in the NdeI and XbaI sites of pBAD24; Amp^r	(Kraxenberger, 2011)
pUT18	Expression vector, Amp^r	(Karimova, 2005)
pUT18C	Expression vector, Amp^r	(Karimova, 2005)
pKT25	Expression vector, Kan^r	(Karimova, 2005)
pKNT25	Expression vector, Kan^r	(Karimova, 2005)
pUT18C-zip	Control plasmid, N-terminal CyaA-T18-yeast leucine-zipper fusion, Amp^r	(Karimova, 2005)
pKT25-zip	Control plasmid, N-terminal CyaA-T25-yeast leucine-zipper fusion, Kan^r	(Karimova, 2005)
pUT18-*yehU* and pUT18-*ypdA*	*yehU*, *ypdA* cloned in XbaI and BamHI sites of pUT18 resulting in N-terminal CyaA-T18-protein fusions	This work
pUT18C-*yehT* and pUT18C-*ypdB*	*yehT*, *ypdB* cloned in XbaI and BamHI sites of pUT18C resulting in C-terminal CyaA-T18-protein fusions	This work
pKT25-*yehS*	*yehS* cloned in XbaI and BamHI sites of pKT25 resulting in C-terminal CyaA-T25-protein fusions	This work

Oligonucleotide	**5'- sequence - 3'**
YehT+A XbaI sense	CCTCTAGAAATGATTAAAGTCTTAATTGTC
YehT+CC BamHI antisense	AAGGATCCCCCAGGCCAATCGCCTCTTTTAA
YpdB+A XbaI sense	GGTCTAGAAGTGAAAGTCATCATTGTTGAA
YpdB+CC BamHI antisense	TCGGATCCCCAAGATGCATTAACTGGCGAAA
YpdA+A XbaI sense	CCTCTAGAAGTGCACGAAATATTCAACATG
YpdA BamHI antisense	TTGGATCCTCAAAGCAATAACGTAGCCTG
YehU+A XbaI sense	GGTCTAGAAATGTACGATTTTAATCTGGTG
YehU BamHI as neu	AAGGATCCTCATGCCTCGTCCCTCCA
YehS+A XbaI sense	TTTCTAGAAATGCTAAGTAACGATATTCTG
YehS+CC BamHI antisense	TTGGATCCCCGCCTTTTTTCACATGCTG
UPyehS-228 sense	TTCTCAATGGCCTGATTTATGAAAAACGCGGCAAGGATGAGTCTGCTCCGTAATACGACTCACTATAGGGCTC
DOWNyehS-229 antisense	AATTTTTTCAGCACGATGTTGTTATTAATGCGACGTTCCGGCTCCAGTGCAATTAACCCTCACTAAAGGGCGC

5.3 Results

In order to get further insights into the complex LytS/LytTR mediated signal transduction network of *E. coli* we analyzed several neighboring genes of *yehUT* and *ypdABC* respectively. We identified with *yehS* (adjacent to the *yehUT* operon) a protein of unknown function. YehS consists of 156 amino acids (predicted molecular weight of 18.0 kDa) and displays a duplication of the domain DUF1456. Cell fractionation experiments confirmed that 6His-YehS is a soluble protein localized in the cytoplasm. Purification (Fig. 5.2 A) and consecutive size exclusion chromatography (Fig. 5.2 B) was performed to determine further properties of 6His-YehS. In cooperation with Dr. Gregor Witte from the Hopfner group (Gene Center, Ludwig-Maximilians-University, München) SEC/RALS analyses for 6His-YehS displayed a monomeric state and an estimated molecular weight of 17.0 kDa. In addition purified 6His-YehS contained unexpectedly high amounts of nucleic acids (A_{260}/A_{280}= 0.82).

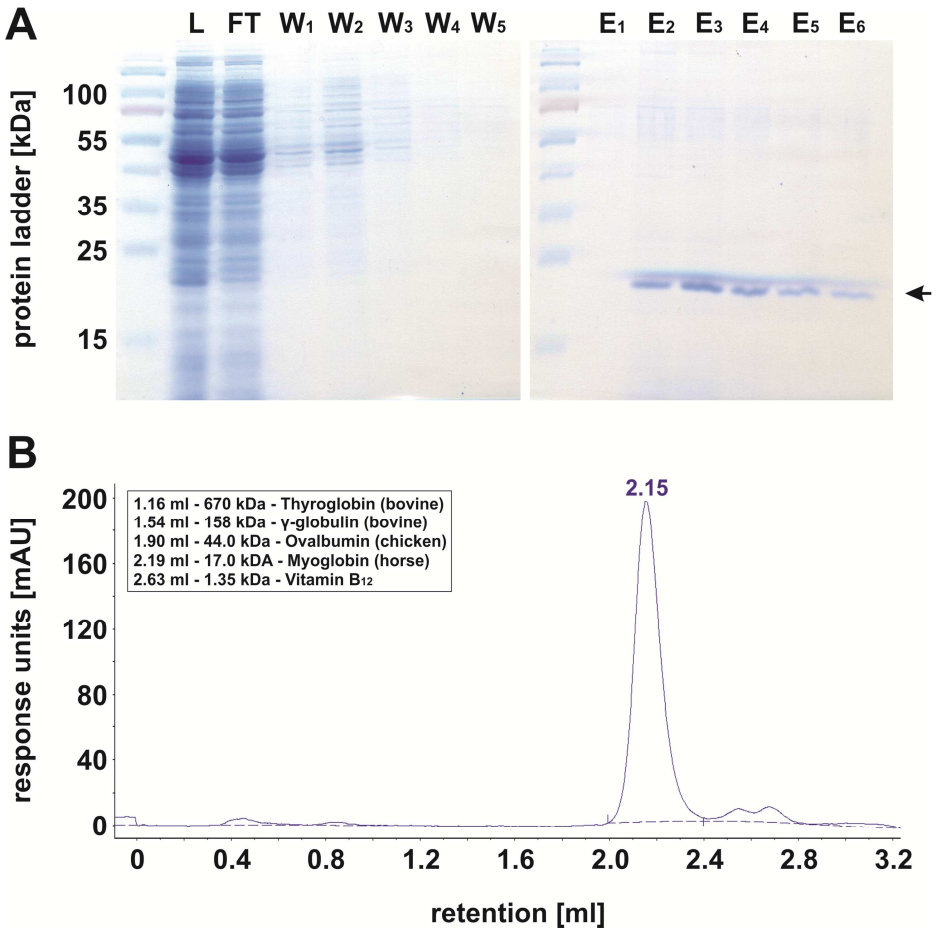

Fig. 5.2 Purification and size-exclusion chromatography for 6His-YehS. A) The purification of 6His-YehS is shown. Individual steps were monitored on SDS-PAGE and visualized via Coomassie staining. The arrow marks 6His-YehS. (L) Bacterial Lysate, (FT) Flow-through, (W) Wash, (E) Eluate. B) A_{280} absorption profile on size-exclusion chromatography Superdex 200 increase 5/150GL column. Retention peak at 2.15 ml marks 6His-YehS.

To validate the influence of YehS on LytS/LytTR mediated target gene expression transcriptional luciferase reporter fusions were tested under inducing conditions. Deletion of *yehS* as well as plasmid based overexpression led to the same observations: a decrease in both (YehU/YehT mediated) *yjiY*- and (YpdA/YpdB mediated) *yhjX* expression (Fig. 5.3 A, B). Since prior studies on *yehS* expression showed no significant alterations within different bacterial growth phases (Fried, 2012), this observation raised the possibility of direct physical contacts between YehS and the LytS/LytTR signaling compounds. To uncover possible interactions between the proteins of interest, we screened all combinations using the bacterial adenylate cyclase two-hybrid system. The yeast leucine zipper-fusion constructs zip-T18 and T25-zip (Karimova, 2005) were used as positive controls (3,000 Miller units, data not shown). For YehS we found an interaction with both histidine kinases (YehU, YpdA) as well as an interaction with both response regulators (YehT-D54E, YpdB) (Fig. 5.3 C). Further studies suggested that the GAF-domain within the histidine kinases might display the interactive interface (Fig. 5.3 C). An interaction between two YehS molecules could not be observed (Fig. 5.3 C), whereas an intramolecular assembly between both DUF1456 domains displayed positive results (Fig. 5.3 D). Based on amino acid substitutions and truncations an initial characterization of YehS suggested a functional interconnectivity between its structural properties (Fig. 5.3 D) and the observed effect on Lyts/LytTR target gene expression (Dörner, 2013).

5.4 Discussion

The role of YehS in LytS/LytTR mediated signal transduction is still unclear. Based on our current observations a coupling function is favored. In this scenario YehS is necessary to mediate the histidine kinase – response regulator interplay to facilitate signal transduction. This idea is supported by the fact that YehS binds both histidine kinases and response regulators. The coupling protein CheW in *E. coli* chemotaxis consists of 167 amino acids (18.1 kDA and provides a physical coupling of CheA to the MCPs allowing regulated phosphotransfer to the CheY and CheB proteins (Li & Hazelbauer, 2011, Underbakke *et al.*, 2011). Interestingly deletion and overexpression of *cheW* also displayed the same chemotactic behavior (Sanders *et al.*, 1989) possibly by disrupting the normal formation of receptor complexes (Cardozo *et al.*, 2010). Likewise YehS might act as a coupling protein for LytS/LytTR mediated signal transduction. In addition it might be conceivable that binding of a small intracellular signaling molecule, like ppGpp or c-di-GMP (Camilli & Bassler, 2006), could provide further regulatory input and hence link LytS/LytTR mediated signal transduction to metabolic or energetic processes.

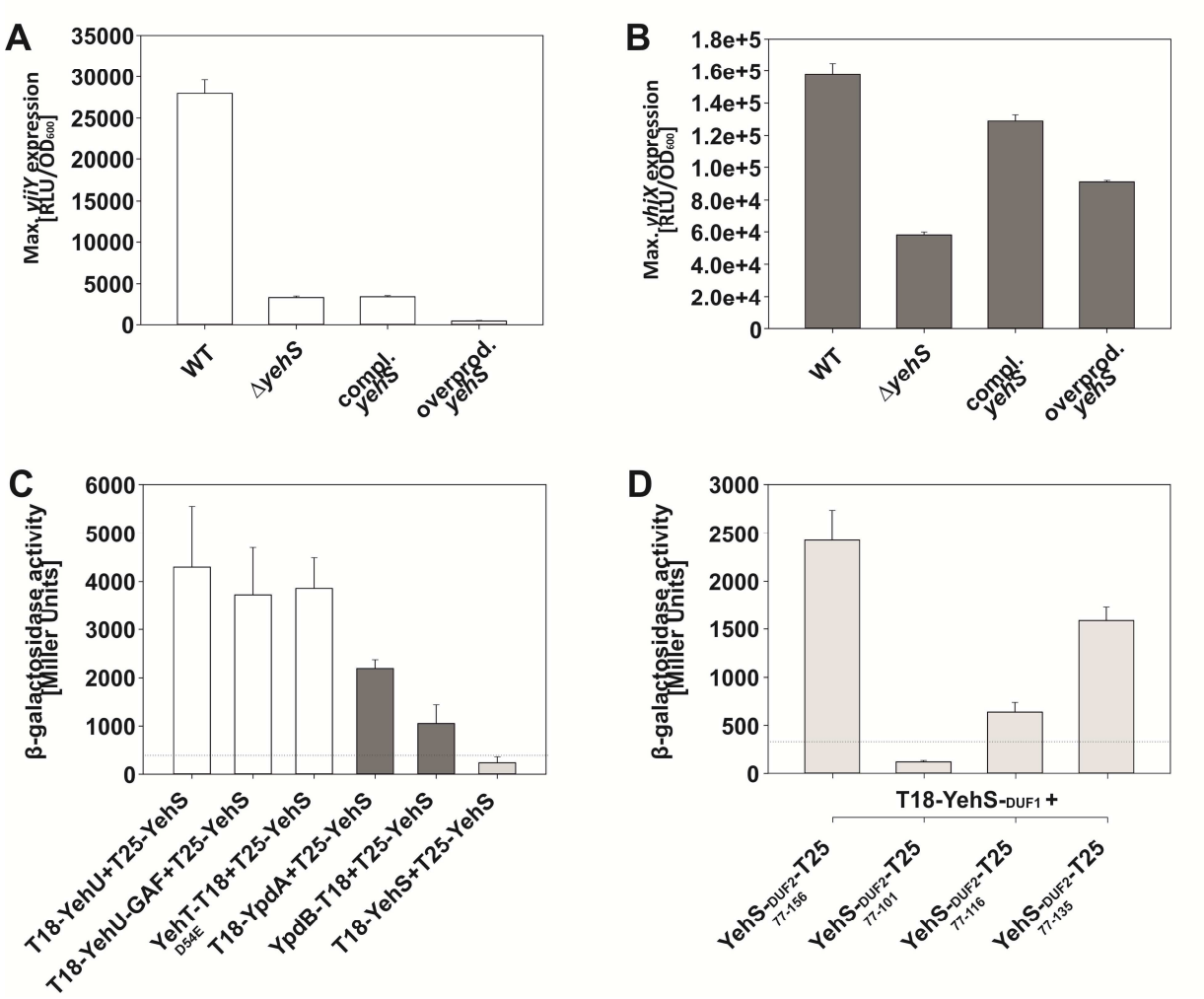

Fig. 5.3 Influence of YehS on LytS/LytTR mediated signal transduction in *E. coli*. Cells were cultivated in M9 minimal medium with inducing conditions for either *yjiY* (0.4 % casamino acids) or *yhjX* (20 mM pyruvate). Optical density (OD_{600}) and luminescence were continuously monitored. Bars indicate the influence of *yehS* deletion or overexpression on maximal A) *yjiY* or B) *yhjX* expression respectively. C) Based on bacterial two-hybrid analyses several interactions of YehS with the HKs and RRs of both LytS/LytTR TCSs were identified. The activity of cells expressing the T25 and T18 fragments on their own was defined as the threshold activity for interaction and is indicated by the dotted line. D) Intramolecular interactions for YehS DUF domain 1 and 2 as well as corresponding truncations in YehS-DUF2 were analyzed in vivo.

5.5 References for Chapter 5

Behr, S., L. Fried & K. Jung, (2014) Identification of a novel nutrient-sensing histidine kinase/response regulator network in *Escherichia coli*. *J. Bacteriol.*: JB. 01554-01514.

Blattner, F. R., G. Plunkett, III, C. A. Bloch, N. T. Perna, V. Burland, M. Riley, J. Collado-Vides, J. D. Glasner, C. K. Rode, G. F. Mayhew, J. Gregor, N. W. Davis, H. A. Kirkpatrick, M. A. Goeden, D. J. Rose, B. Mau & Y. Shao, (1997) The complete genome sequence of *Escherichia coli* K-12. *Science* **277**: 1453-1462.

Buelow, D. R. & T. L. Raivio, (2010) Three (and more) component regulatory systems - auxiliary regulators of bacterial histidine kinases. *Mol. Microbiol.* **75**: 547-566.

Camilli, A. & B. L. Bassler, (2006) Bacterial small-molecule signaling pathways. *Science* **311**: 1113-1116.

Cardozo, M. J., D. A. Massazza, J. S. Parkinson & C. A. Studdert, (2010) Disruption of chemoreceptor signalling arrays by high levels of CheW, the receptor–kinase coupling protein. *Mol. Microbiol.* **75**: 1171-1181.

Cole, C., J. D. Barber & G. J. Barton, (2008) The Jpred 3 secondary structure prediction server. *Nucleic Acids Res.* **36**: W197-W201.

Dörner, K., (2013) Untersuchungen zur physiologischen Funktion von YehS in der LytS/LytTR-vermittelten Signaltransduktion von *Escherichia coli*. *Bachelor Thesis*: Ludwig-Maximilians-Universität München.

Finn, R. D., J. Mistry, J. Tate, P. Coggill, A. Heger, J. E. Pollington, O. L. Gavin, P. Gunasekaran, G. Ceric, K. Forslund, L. Holm, E. L. L. Sonnhammer, S. R. Eddy & A. Bateman, (2010) The Pfam protein families database. *Nucleic Acids Res.* **38**: D211-D222.

Franceschini, A., D. Szklarczyk, S. Frankild, M. Kuhn, M. Simonovic, A. Roth, J. Lin, P. Minguez, P. Bork & C. von Mering, (2013) STRING v9. 1: protein-protein interaction networks, with increased coverage and integration. *Nucleic Acids Res.* **41**: D808-D815.

Fried, L., (2012) Insights into stimulus perception, target gene expression and network formation of LytS/LytTR-like histidine kinase response regulator systems in *Escherichia coli*. *Dissertation*: Ludwig-Maximilians-Universität München.

Fried, L., S. Behr & K. Jung, (2013) Identification of a target gene and activating stimulus for the YpdA/YpdB histidine kinase/response regulator system in *Escherichia coli*. *J. Bacteriol.* **195**: 807-815.

Guzman, L., D. Belin, M. Carson & J. Beckwith, (1995) Tight regulation, modulation, and high-level expression by vectors containing the arabinose P_{BAD} promoter. *J. Bacteriol.* **177**: 4121-4130.

Heermann, R., A. Weber, B. Mayer, M. Ott, E. Hauser, G. Gabriel, T. Pirch & K. Jung, (2009) The universal stress protein UspC scaffolds the KdpD/KdpE signaling cascade of *Escherichia coli* under salt stress. *J. Mol. Biol.* **386**: 134-148.

Heermann, R., T. Zeppenfeld & K. Jung, (2008) Simple generation of site-directed point mutations in the *Escherichia coli* chromosome using Red®/ET® Recombination. *Microb. Cell Fact.* **7**: 14.

Jung, K., L. Fried, S. Behr & R. Heermann, (2012) Histidine kinases and response regulators in networks. *Curr. Opin. Microbiol.* **15**: 118-124.

Karimova, G., Ladant, D., (2005) A bacterial two-hybrid system based on a Cyclic AMP signaling cascade. In: In Protein–Protein Interactions. E. Golemis, ed. Cold Spring Harbor, (ed). NY: Cold Spring Harbor Laboratory Press, pp. 499-515.

Karimova, G., J. Pidoux, A. Ullmann & D. Ladant, (1998) A bacterial two-hybrid system based on a reconstituted signal transduction pathway. *Proc. Natl. Acad. Sci. U.S.A.* **95**: 5752-5756.

Keseler, I. M., C. Bonavides-Martínez, J. Collado-Vides, S. Gama-Castro, R. P. Gunsalus, D. A. Johnson, M. Krummenacker, L. M. Nolan, S. Paley, I. T. Paulsen, M. Peralta-Gil, A. Santos-Zavaleta, A. G. Shearer & P. D. Karp, (2009) EcoCyc: A comprehensive view of *Escherichia coli* biology. *Nucleic Acids Res.* **37**: D464-D470.

Kox, L. F., M. M. Wosten & E. A. Groisman, (2000) A small protein that mediates the activation of a two-component system by another two-component system. *EMBO J.* **19**: 1861-1872.

Kraxenberger, T., (2011) Zur Funktion des Sensor-Histidinkinase/Antwortregulator Systems YehU/YehT in *Escherichia coli*. *Dissertation*: Ludwig-Maximilians-Universität München.

Kraxenberger, T., L. Fried, S. Behr & K. Jung, (2012) First insights into the unexplored two-component system YehU/YehT in *Escherichia coli*. *J. Bacteriol.* **194**: 4272-4284.

Krell, T., J. Lacal, A. Busch, H. Silva-Jiménez, M.-E. Guazzaroni & J. L. Ramos, (2010) Bacterial sensor kinases: Diversity in the recognition of environmental signals. *Annu. Rev. Microbiol.* **64**: 539-559.

Laemmli, U. K., (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227**: 680-685.

Li, M. & G. L. Hazelbauer, (2011) Core unit of chemotaxis signaling complexes. *Proc. Natl. Acad. Sci. USA* **108**: 9390-9395.

Lowry, O. H., N. J. Rosebrough, A. L. Farr & R. J. Randall, (1951) Protein measurement with the folin phenol reagent. *J. Biol. Chem.* **193**: 265-275.

McGuffin, L. J., K. Bryson & D. T. Jones, (2000) The PSIPRED protein structure prediction server. *Bioinformatics* **16**: 404-405.

Meselson, M. & R. Yuan, (1968) DNA restriction enzyme from *E. coli*. *Nature* **217**: 1110-1114.

Miller, J. H., (1992) *A short course in bacterial genetics: a laboratory manual and handbook for Escherichia coli and related bacteria,* p. 875. Cold Spring Harbor (N.Y.): Cold Spring Harbor Laboratory Press, Plainview, N.Y.

Rauschmeier, M., V. Schüppel, L. Tetsch & K. Jung, (2014) New insights into the interplay between the lysine transporter LysP and the pH sensor CadC in *Escherichia coli*. *J. Mol. Biol.* **426**: 215-229.

Sanders, D., B. Mendez & D. Koshland, (1989) Role of the CheW protein in bacterial chemotaxis: overexpression is equivalent to absence. *J. Bacteriol.* **171**: 6271-6278.

Shin, D., E.-J. Lee, H. Huang & E. A. Groisman, (2006) A positive feedback loop promotes transcription surge that jump-starts Salmonella virulence circuit. *Science* **314**: 1607-1609.

Studier, F. W. & B. A. Moffatt, (1986) Use of bacteriophage T7 RNA polymerase to direct selective high-level expression of cloned genes. *J. Mol. Biol.* **189**: 113-130.

Tetsch, L., C. Koller, I. Haneburger & K. Jung, (2008) The membrane-integrated transcriptional activator CadC of *Escherichia coli* senses lysine indirectly via the interaction with the lysine permease LysP. *Mol. Microbiol.* **67**: 570-583.

Underbakke, E. S., Y. Zhu & L. L. Kiessling, (2011) Protein footprinting in a complex milieu: identifying the interaction surfaces of the chemotaxis adaptor protein CheW. *J. Mol. Biol.* **409**: 483-495.

6 Manuscript: A novel allosteric DNA-binding mechanism of a bacterial response regulator

Stefan Behr, Ralf Heermann, and Kirsten Jung

From Munich Center for Integrated Protein Science (CIPSM) at the Department of Microbiology, Ludwig-Maximilians-Universität München, 82152 Martinsried, Germany

***Running title:** DNA binding kinectics of the LytTR response regulator YpdB

To whom correspondence should be addressed: Dr. Kirsten Jung, Ludwig-Maximilians-Universität München, Department Biologie I, Bereich Mikrobiologie, Großhaderner Str. 2-4, 82152 Martinsried, Germany. Phone: +49-89-2180-74500; Fax: +49-89-2180-74520; E-mail: jung@lmu.de

Keywords: LytTR response regulator, Protein-DNA-interaction

A novel allosteric DNA-binding mechanism of a bacterial response regulator

Stefan Behr, Ralf Heermann, and Kirsten Jung

From Munich Center for Integrated Protein Science (CIPSM) at the Department of Microbiology, Ludwig-Maximilians-Universität München, 82152 Martinsried, Germany

***Running title:** DNA binding kinectics of the LytTR response regulator YpdB

To whom correspondence should be addressed: Dr. Kirsten Jung, Ludwig-Maximilians-Universität München, Department Biologie I, Bereich Mikrobiologie, Großhaderner Str. 2-4, 82152 Martinsried, Germany. Phone: +49-89-2180-74500; Fax: +49-89-2180-74520; E-mail: jung@lmu.de

Keywords: LytTR response regulator, Protein-DNA-interaction

Highlights

- Based on a calibration-free concentration analysis (CFCA) real-time association- and dissociation events provide superior experimental evidence for DNA-binding and affinities
- Thereby the LytTR-like RR YpdB-D53E displayed a hitherto unknown allosteric two-step DNA binding mechanism, which might be necessary to generate a dynamic pulse expression control for substantial cellular mechanisms.

Abstract

Bacterial two-component systems (TCSs) link environmental changes to cellular adaptation mechanisms. Thereby sensor histidine kinases (HKs) undergo autophosphorylation upon stimulus perception. The transfer of the phosphoryl group to the corresponding response regulator (RR) mediates cellular adaptations predominantly via alterations in gene expression. The protein family of LytS/LytTR-like TCSs is very wide-spread and is involved the regulation of various bacterial pathogenicity mechanisms. In this study we focused on the DNA-binding mechanism of the LytTR-like RR YpdB of *Escherichia coli*. We determined binding kinetics for YpdB and the phosphorylated mimetic YpdB-D53E via surface plasmon resonance spectroscopy measurements. Based on nucleotide substitutions we were able to uncover the mechanistic nature of a hitherto unknown allosteric two-step binding event. Upon the combination of RR

phosphorylation, DNA-binding and rapid promoter clearance, we propose an enhanced transcriptional control, which might be necessary to create a pulsed expression mechanism.

Introduction

Two-component signal transduction systems (TCSs) represent the predominant bacterial mechanism to sense and respond to environmental conditions. TCSs comprise an often membrane-integrated sensor histidine kinase (HK), which perceives a stimulus and a cytoplasmic response regulator (RR) with DNA-binding affinity [1]. While signal perception entails activation and auto-phosphorylation of the HK, a subsequent phosphotransfer reaction to its cognate RR induces cellular adaptations.

Based on their structural properties TCSs are classified into different families. The LytS/LytTR-like family of TCSs is wide-spread among human and plant pathogenic bacteria and regulates a variety of virulence- and virulence-associated factors, like toxin production in *Staphylococcus aureus* [2], natural competence of *Streptococcus pneumonia* [3] or the biosynthesis of extracellular polysaccharides in *Pseudomonas aeruginosa* [4; 5]. All RR of the LytTR-family share an uncommon DNA-binding domain. A conserved 10 stranded β fold mediates DNA binding via three elongated β sheets, while highly variable residues within the connecting loop regions determine binding specificity [6]. Hence LytTR RRs share no common consensus binding sequence. In contrast to that, almost all LytTR RR display a similar DNA-binding pattern of direct or inverse repeats (between 9 and 11 nucleotides in length) separated by 11 to 13 spacer nucleotides [7; 8; 9]. Furthermore, several studies indicate an influence of surrounding DNA structure on RR binding [10; 11; 12; 13]. In *Escherichia coli* the LytS/LytTR-like TCS YpdA/YpdB has been described recently [13]. The HK YpdA responds to extracellular pyruvate and activates its cognate RR YpdB, which in turn induces *yhjX* expression. The gene *yhjX* is the only direct target gene of YpdB and encodes a putative transport protein of the major facilitator superfamily. The YpdA/YpdB TCS is part of a complex nutrient-sensing network [14] and is supposed to contribute to nutrient scavenging before cells enter stationary phase [13]. The RR YpdB is characterized by an N-terminal receiver domain, with a highly conserved aspartate at position 53, and a C-terminal LytTR effector domain with DNA-binding affinity. Conversion of aspartate 53 to glutamate (YpdB-D53E) was shown to result in a constitutively activated protein with a phosphorylation independent phenotype of constant *yhjX* expression [13]. The LytTR-like RR YpdB binds to a direct repeat (M1 and M2) of a 10 bp motif (GGCATTTCAT) with an 11 bp spacer region.

In this study we focused on the molecular mechanisms behind LytTR-mediated gene expression. Based on surface plasmon resonance spectroscopy measurements we closely analyzed binding of YpdB and the phosphorylated mimetic YpdB-D53E to its binding site in *yhjX* promoter DNA. Thereby substitutions of motifs M1 or M2 within this binding site revealed different affinities for RR-binding and suggest a hitherto novel concept of allosteric interaction.

Materials and Methods

Molecular biological techniques and protein purification.

E. coli strain BL21(DE3) [15] was transformed with pBAD24-*ypdB* or pBAD24-*ypdB*-D53E respectively [13]. Overproduction of 6His-tagged proteins and subsequent purification via Ni-NTA-affinity chromatography was performed as described before [12] and 95% pure as judged by SDS-PAGE [16]. To address structural properties, a size exclusion chromatography was performed on a calibrated Superdex 200 Increase GL

HR10/300 column (GE Healthcare) in the presence of 50 mM Tris/HCl pH 7.6, 10% (v/v) glycerol, 150 mM NaCl, 2 mM DTT.

5'-biotinylated oligonulceotides and their complementary non-biotinylated strands (flanked by four guanine-cytosine nucleotide bonds) (Table 6.1) were ordered by Sigma Aldrich and independently annealed by heating equimolar samples (95°C for 5 minutes) and subsequent cooling down at room temperature. Resultant biotinylated DNA double stands contained motif substitutions with purine to pyrimidine conversions (and vice versa).

Table 6.1: Oligonucleotides annealed for SPR binding experiments on SA sensor chip

Name	**5' – sequence -3'**
[BTN] *yhjX* YpdB bs sense	[BTN]GGGGCGCGTCATTCATTCCTGAACTAAGGCATTTCATTCCGTTCTGATGGCATTTCATGCCGGGGG
yhjX YpdB bs antisense	CCCCCGGCATGAAATGCCATCAGAACGGAATGAAATGCCTTAGTTCAGGAATGAATGACGCGCCCC
[BTN] *yhjX* YpdB M1 bs sense	[BTN]GGGGCGCGTCATTCATTCCTGAACTAAttacgggacgTCCGTTCTGATGGCATTTCATGCCGGGGG
yhjX YpdB M1 bs antisense	CCCCCGGCATGAAATGCCATCAGAACGGAcgtcccgtaaTTAGTTCAGGAATGAATGACGCGCCCC
[BTN] *yhjX* YpdB M2 bs sense	[BTN]GGGGCGCGTCATTCATTCCTGAACTAAGGCATTTCATTCCGTTCTGATttacgggacgGCCGGGGG
yhjX YpdB M2 bs antisense	CCCCCGGCcgtcccgtaaATCAGAACGGAATGAAATGCCTTAGTTCAGGAATGAATGACGCGCCCC
[BTN] *yjiY* YehT bs sense	[BTN]GGGGCCTTTGCCGCTCAACCGCAAAACTGACCGCTTACATCCCTAAAATAACCACTCAGTTAGGGG
yjiY YehT bs antisense	CCCCTAACTGAGTGGTTATTTTAGGGATGTAAGCGGTCAGTTTTGCGGTTGAGCGGCAAAGGCCCC

Surface Plasmon Resonance (SPR-) Sprectroscopy

SPR assays were performed in a Biacore T200 using carboxymethyl dextran sensor chips pre-coated with streptavidin (SA Sensor Chip Series S). All experiments were carried out at a constant temperature of 25°C and using HBS-EP buffer (10 mM HEPES pH 7.4; 150 mM NaCl; 3 mM EDTA; 0.005 % (v/v) detergent P20) as running buffer. Before immobilizing the DNA fragments, the chips were equilibrated by three injections using 1 M NaCl/50 mM NaOH at a flow rate of 10 µl/min. Then, 10 nM of the respective double-stranded biotinylated DNA fragment was injected using a contact time of 420 sec and a flow rate of 10 µl/min. As a final wash step, 1 M NaCl/50 mM NaOH/50% (v/v) isopropanol was injected. Approximately 100-200 RU of each respective DNA fragment were captured onto each flow cell. The interaction kinetics of YpdB and YpdB-D53E with the respective DNA fragment were all performed in HBS-EP buffer at 25°C at a flow rate of 30 µl/min. The proteins were diluted in HBS-EP buffer and passed over all flow cells in different concentrations (1 nM-50 nM) using a contact time of 180 sec followed by a 300 sec dissociation time before the next cycle started. After each cycle the surface was regenerated by injection of 2.5 M NaCl for 30 sec at 60 µl/min flow rate followed by a second regeneration step by injection of 0.5% (w/v) SDS for 30 sec at 60 µl/min. All experiments were performed at 25°C. Sensorgrams were recorded using the Biacore T200 Control software 1.0 and analyzed with the Biacore T200 Evaluation software 1.0. The surface of flow cell 1 was coated with *yjiY*-DNA (no binding of YpdB) and used to obtain blank sensorgrams for subtraction of bulk refractive index background. The

referenced sensorgrams were normalized to a baseline of 0. Peaks in the sensorgrams at the beginning and the end of the injection emerged from the runtime difference between the flow cells of each chip.
Calibration-free concentration analysis (CFCA) was performed using a 5 µM sample solution of YpdB-D53E (calculated from classical determination of protein concentration), which was stepwise diluted 1:2, 1:5, 1:10, and 1:20. Each protein dilution was two-times injected, one at 5 µl/min as well as 100 µl/min flow rate. On the active flow cell $P_{yhjX\text{-}M1}$-DNA was used for YpdB-D53E-binding, and P_{yhjX}-DNA on the reference cell. CFCA basically relies on mass transport, which is a diffusion phenomenon that describes the movement of molecules between the solution and the surface. The CFCA therefore relies on the measurement of the observed binding rate during sample injection under partially or complete mass transport limited conditions. Overall, the initial binding rate (dR/dt) is measured at two different flow rates dependent on the diffusion constant of the protein. The diffusion coefficent of YpdB-D53E was calculated using the Biacore diffusion constant calculator and converter webtool (https://www.biacore.com/lifesciences/Application_Support/online_support/Diffusion_Coefficient_Calculator/index.html), whereas a globular shape of the protein was assumed. The diffusion coefficent of YpdB-D53E was determined as $D=9.94x10^{-11}$ m^2/s. The initial rates of those dilutions that differed in a factor of at least 1.5 were considered for the calculation of the „active“ concentration, which was determined as $5x10^{-8}$M (1% of the total protein concentration) for YpdB-D53E. The „active“ protein concentration was further used for calculation of the binding kinetic constants.

Interaction Map® (IM) analysis

IM calculations were performed on the Ridgeview Diagnostic Server (Ridgeview Diagnostics, Uppsala, Sweden). For this purpose, the SPR sensorgrams were exported from the Biacore T200 Evaluation Software 1.0 as *.txt files and imported into the TraceDrawer Software 1.5 (Ridgeview Instruments, Uppsala, Sweden). IM files were created using the IM tool within the software, generating files that were sent via e-mail to the server (im@ridgeviewdiagnostics.com) where the IM calculations were performed [17]. The result files were then evaluated for spots in the TraceDrawer 1.5 software, and the IM spots were quantified.

Results and Discussion

To determine binding kinetics for YpdB and YpdB-D53E to their corresponding binding motifs within *yhjX* promoter-DNA, we performed surface plasmon resonance (SPR-) spectroscopy measurements. For that purpose, 6His-YpdB and 6His-YpdB-D53E, respectively, were overproduced and purified via Ni-NTA-affinity chromatography. Size exclusion chromatography showed that both full-length RR proteins are exclusively monomeric in solution and hence suitable for SPR measurements (data not shown).

As first step, 5'-biotinylated double strand DNA (displaying the complete YpdB binding site) was immobilized on a SA Sensor Chip. A calibration-free concentration analysis (CFCA) (see Materials and Methods for detail) was used for determination of the "active" protein concentration in YpdB-D53E, i.e. protein that binds the DNA fragment (Supplemental material), and this amount was used for calculation of "active" YpdB protein also. For the determination of binding-kinetics, increasing concentrations (10-50 nM) of YpdB or YpdB-D53E were passed over the chip surface using a contact time of 180 sec (association), followed by a 300 sec dissociation phase. It could be observed that binding of YpdB to the *yhjX* promoter-DNA (P_{yhjX}) was approximately 5-times lower compared to YpdB-D53E, revealing that the latter one is a perfect variant mimicking the active state of the RR (Figs. 6.1 A and B).

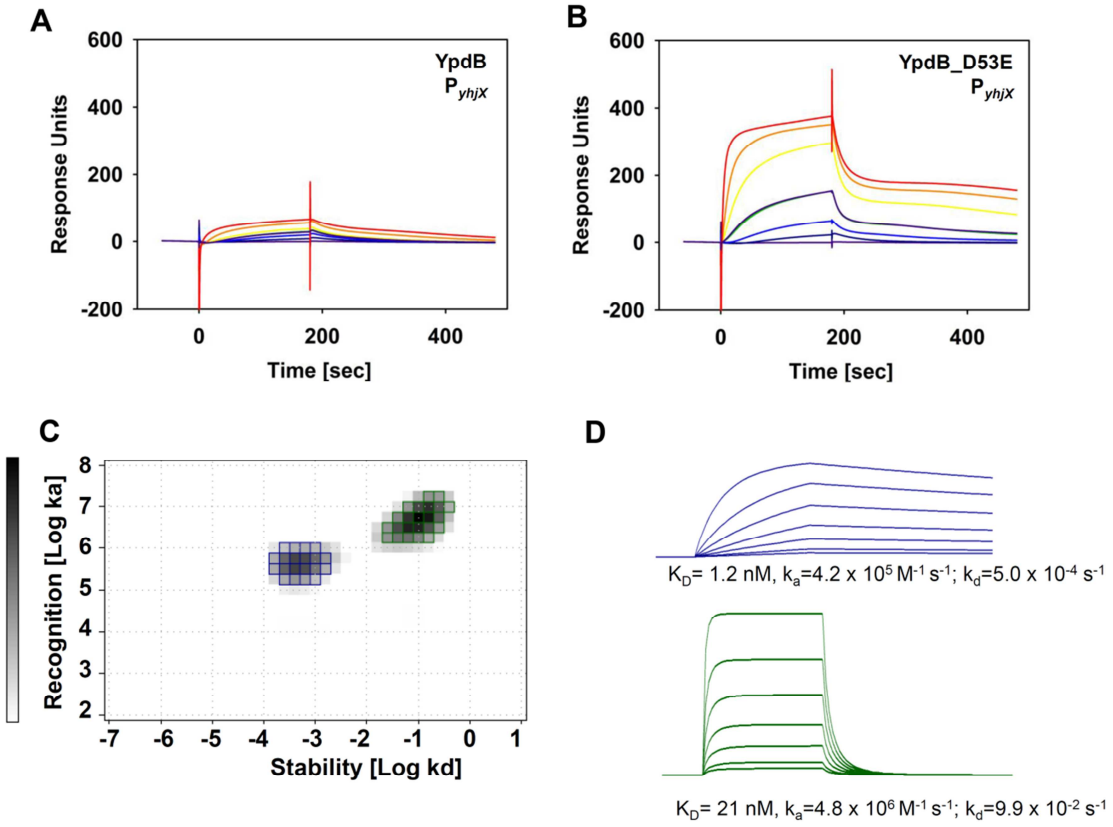

Figure 6.1: Binding of YpdB and YpdB-D53E to the promoter region of *yhjX* (P_{yhjX}). (A and B) SPR analyses. The Biotin-labeled DNA fragment comprising P_{yhjX} was captured onto a SA sensor chip, and solutions of 1 nM (violet line), 2.5 nM (blue line), 5 nM (dark blue and green line (internal reference)), 10 nM (yellow line), 20 nM (orange line) and 50 nM (red line), respectively, of each of purified YpdB (A) or YpdB-D53E (B) was passed over the chip. (C) IM analyses of YpdB-D53E-P_{yhjX} interaction. The green and the blue spots represent both the YpdB-D53E interaction with the DNA, indicating that two binding sites with different affinities exists in the *yhjX*-promoter region (D) Calculated sensorgrams for YpdB-D53E interactions with the P_{yhjX}. The calculated K_D values as well as the ON/OFF-

rates for each interaction are indicated below the respective sensorgram.

In addition to this, none of the sensorgrams for YpdB-D53E followed the calculated kinetic assumptions of a 1:2 binding event. This indicates that the interaction of YpdB-D53E to its corresponding binding sites in *yhjX* promoter does not reflect a monovalent binding mechanism and raised the possibility of multiple binding events. To calculate reliable binding constants and kinetic parameters, a computational approach was chosen to analyze the sensorgrams. The measured curves can be approximated to the sum of individual binding curves, each representing a monovalent interaction [18] with a unique combination of association rate k_a (ON-rate) and dissociation rate k_d (OFF-rate) (and consequently a real equilibrium dissociation constant $K_D = k_d/k_a$).

Therefore, we calculated a so called interaction map (IM) for YpdB-D53E sensorgrams to determine and quantify the individual binding events represented by the curves. The algorithm splits the experimental SPR data set to several theoretical monovalent binding curves and spots the binding curves that, summed up, best fit the experimental data. By plotting the association rate k_a and the dissociation rate k_d within a two-dimensional distribution, it is possible to display heterogeneous binding data as a map where each peak corresponds to one component that contributes to the cumulative binding curve [17]. In case that these interaction events have almost similar on- and off-rates, no separate but fused peaks will appear.

Based on the SPR sensorgrams of YpdB-D53E, interaction map analyses identified two clearly separated peak values (Fig. 6.1 C). The first peak (blue) with a peak weight of 33.5% displays an ON-rate of 4.2 x 10^5 M^{-1} s^{-1} and an OFF-rate of 5.0 x 10^{-4} s^{-1} resulting in a K_D value of 1.2 nM (Figs. 6.1 C and D, upper panel). The second peak (green) with a peak weight of 51.6% displays a 10-times higher ON-rate. The OFF-rate for this interaction was determined to be 9.9 x 10^{-2} s^{-1} (50-fold higher in comparison to first peak (blue)), which hence replays an interaction with a calculated K_D value of 21 nM (Figs. 6.1 C and D, lower panel). Based on the 'active' protein fraction from the CFCA the average K_D value was determined to be 13.2 nM, which is more than 10-times higher compared to our results from gel retardation experiments [13]. Besides the real-time determination of association- and dissociation events, this CFCA analysis displays another superior advantage of SPR measurements for DNA binding experiments.

In the following experiments we focused on the assignment of these two interaction events. YpdB-D53E binds to a well-characterized direct repeat of the nucleotide motif GGCATTTCAT in P_{yhjX}, hereinafter referred to as motifs M1 and M2 (Fig. 6.2 A). Therefore we annealed two biotinylated DNA fragments comprising nucleotide substitutions (purine to pyrimidine conversions (and vice versa)) for either the first (P_{yhjX_M1}) or the second binding motif (P_{yhjX_M2}).

Binding of YpdB-D53E was completely abolished when motif M1 was substituted (Fig. 6.2 B). However, when motif M2 was substituted YpdB-D53E was still able to bind the DNA fragment (Fig. 6.2 C). In contrast to binding of YpdB-D53E to P_{yhjX}, the sensorgrams for P_{yhjX_M2} display a single interaction event (Fig. 6.2 C). Calculations to generate the corresponding interaction maps confirmed this observation (blue peak – weight of 68.2 %). The additional small peak depicted in green with a peak weight of 3.5% did not reflect a defined binding event and was supposed to correspond to bulk effects. The ON- (3.4 x 10^4 M^{-1} s^{-1}) and OFF-rate (7.8 x 10^{-4} s^{-1}) displayed a K_D value of 23 nM and reflected the binding properties of the first binding curve observed for YpdB-D53E P_{yhjX} interaction (blue, Fig. 6.1 D). Nevertheless it is worth mentioning, that ON-rate of YpdB-D53E was 10-times lower when motif M2 was replaced (in comparison to P_{yhjX}), whereas the OFF-rate remained indistinguishable.

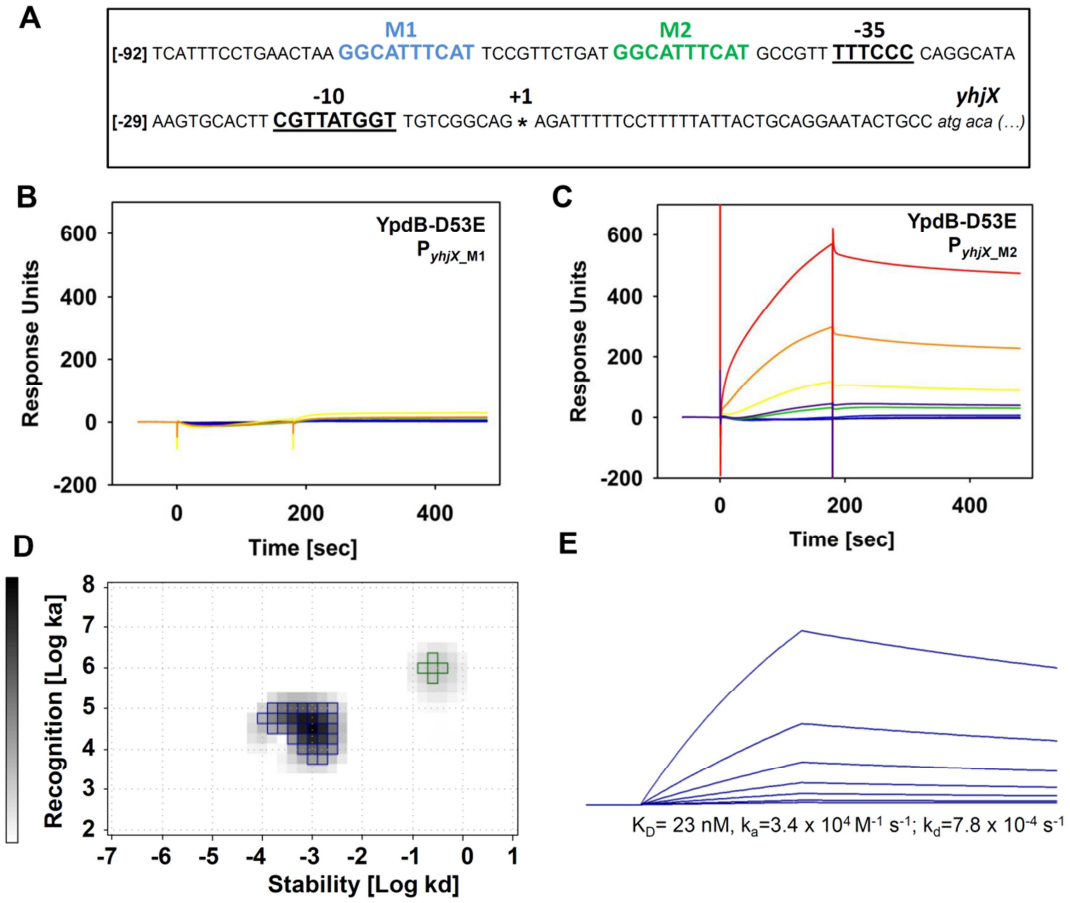

Figure 6.2: Binding of YpdB-D53E to the P_{yhjX_M1} and P_{yhjX_M2}. (A) Schematic presentation of the full length *yhjX*-promoter region. Motifs M1 (blue) and M2 (green) are colored, underlined sequences represent ribosome binding sites. The asterisk marks the translational start site, whereas the *yhjX* gene is written in italics. (B and C) SPR analyses. The biotin-labeled DNA fragments comprising the respective substitutions in motif M1 (P_{yhjX_M1}) (B) or M2 (P_{yhjX_M2}) of the *yhjX* promoter region (C) were captured onto a SA sensor chip, and solutions of 1 nM (violet line), 2.5 nM (blue line), 5 nM (dark blue and green line (internal reference)), 10 nM (yellow line), 20 nM (orange line) and 50 nM (red line), of purified YpdB-D53E was passed over the chip. (D) IM analyses of YpdB-D53E-P_{yhjX_M2} interaction. The blue spot represents the YpdB-D53E interaction with the P_{yhjX_M2}, indicating that only one DNA-binding site for YpdB-D53E exists. The green spot with peak weight of <5% represents no binding event (see text). (E) Calculated sensorgrams for YpdB-D53E interactions with the P_{yhjX_M2}. The calculated K_D value as well as the ON/OFF-rate is indicated below the sensorgram.

Furthermore the calculated interaction peak became more blurred. This indicates a broader deviation for k_a and k_d values, suggesting a decreased interaction stability. Hence the efficient binding of a second RR molecule to motif M2 also seems to influence motif M1 binding stability.

Taken together we could show that both YpdB variants are monomeric in solution. Nevertheless activation of YpdB, mimicked by an aspartate-to-glutamate substitution on position 53, is necessary to specifically bind *yhjX* promoter DNA in a concentration dependent manner. Dimerization of activated RR molecules is a common mechanism in many bacterial signal transduction pathways [1]. Thereby a highly-conserved aspartate residue in the receiver domain of the RR gets phosphorylated and mediates conformational changes, which in turn result in activation. Linkage between activation and dimerization has been described for several RRs from different protein families, as recently for VraR from

Staphlococcus aureus [19] or PhoB from *E. coli* [20]. Furthermore structural studies on isolated receiver domain variants from LytTR-like RR ComE from *Streptococcus pneumoniae* demonstrated activation dependent monomer-to-dimer transition [21].

Our results suggest that the activation of the LytTR RR YpdB is in particularly important for initial DNA binding. This idea is favored by the observation of an allosteric two step binding event: while binding of YpdB-D53E to *yhjX* promoter DNA is completely prevent upon substitution of motif M1, an interaction can still be observed when motif M2 is substituted. This indicates that initial binding of a first RR molecule occurs on motif M1 (Fig. 6.3).

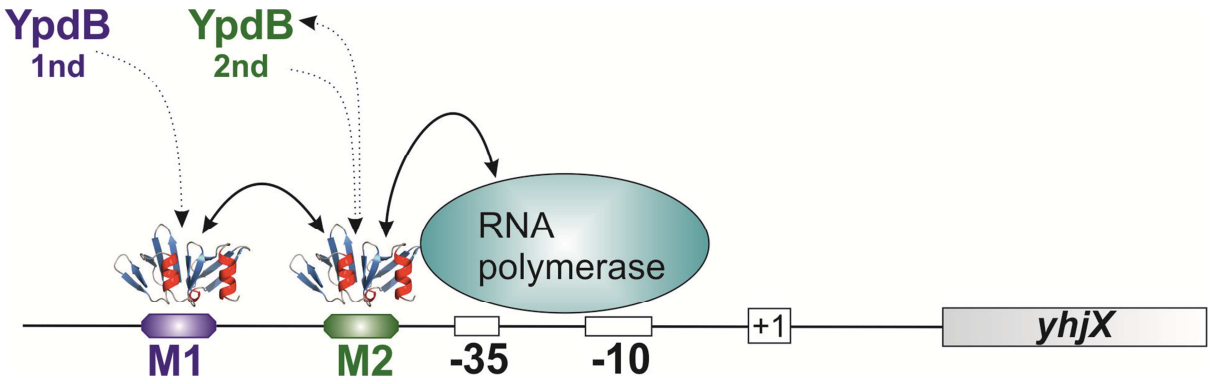

Figure 6.3: Schematic model of the cooperative binding mechanism of YpdB-D53E to the *yhjX* promoter region. Initial binding of YpdB to *yhjX* promoter DNA occurs on motif M1. As a consequence a second YpdB molecule occupies motif M2 and enables RNA polymerase recruitment. The binding properties of YpdB to motif M2 promote rapid motif clearance, which could be necessary to generate a dynamic pulse expression control.

Reduction of the ON-rate (for motif M1 binding) when motif M2 is missing indicates simultaneous stabilization effects of a second RR molecule, which in turn depends on motif M2 availability. Nevertheless occupation of motif M1 is crucial for further DNA-binding of the second RR molecule. The reason for this might be the induction of major conformational changes on DNA structure. Interestingly DNA analyses predicted an average curvature for motif M1 between 4 and 6 degree, whereas motif M2 curvature ranged from 8 and 11 degree [22]. This already indicates major structural differences. Furthermore binding of the second RR molecule could be stabilized by intermolecular interactions as it has been shown lately for KdpE [23]. Nevertheless in earlier studies we could show, that both motifs are essential for gene expression in vivo [13]. Hence also binding of the second RR molecule contributes to further adaptions to induce gene expression. Structural studies on the LytTR-binding domain of AgrA from *S. aureus* revealed strong DNA bending upon binding of two RR molecules [6], which is thought to be essential for the recruitment of RNA-Polymerase. Furthermore binding of YpdB-D53E to motif M2 could also provide the required protein interface for RNA-Polymerase recruitment.

Interestingly the binding properties of YpdB-D53E to motif M2 indicate a high turnover of this interaction. This rapid promoter clearance could function as a mechanism to prevent excessive gene expression and might enable a dynamic pulse expression control in more switch-like behavior [24]. Structural similarities within the promoter regions of LytTR-regulated genes support this idea, in particular since these genes are often participants of tightly regulated processes, like toxin production or pathogenicity.

References

1. Jung, K., Fried, L., Behr, S. & Heermann, R. (2012). Histidine kinases and response regulators in networks. *Curr. Opin. Microbiol.* **15**, 118-24.

2. Bronner, S., Monteil, H. & Prévost, G. (2004). Regulation of virulence determinants in *Staphylococcus aureus*: complexity and applications. *FEMS Microbiol. Rev.* **28**, 183-200.

3. de Saizieu, A., Gardes, C., Flint, N., Wagner, C., Kamber, M., Mitchell, T. J., Keck, W., Amrein, K. E. & Lange, R. (2000). Microarray-based identification of a novel *Streptococcus pneumoniae* regulon controlled by an autoinduced peptide. *J. Bacteriol.* **182**, 4696-703.

4. Lizewski, S. E., Schurr, J. R., Jackson, D. W., Frisk, A., Carterson, A. J. & Schurr, M. J. (2004). Identification of AlgR-regulated genes in *Pseudomonas aeruginosa* by use of microarray analysis. *J. Bacteriol.* **186**, 5672-5684.

5. Morici, L. A., Carterson, A. J., Wagner, V. E., Frisk, A., Schurr, J. R., zu Bentrup, K. H., Hassett, D. J., Iglewski, B. H., Sauer, K. & Schurr, M. J. (2007). Pseudomonas aeruginosa AlgR represses the Rhl quorum-sensing system in a biofilm-specific manner. *J. Bacteriol.* **189**, 7752-7764.

6. Sidote, D. J., Barbieri, C. M., Wu, T. & 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.

7. Cheung, J. K. & Rood, J. I. (2000). The VirR response regulator from *Clostridium perfringens* binds independently to two imperfect direct repeats located upstream of the *pfoA* promoter. *J. Bacteriol.* **182**, 57.

8. Diep, D. B., Myhre, R., Johnsborg, O., Aakra, A. & Nes, I. F. (2003). Inducible bacteriocin production in *Lactobacillus* is regulated by differential expression of the *pln* operons and by two antagonizing response regulators, the activity of which is enhanced upon phosphorylation. *Mol. Microbiol.* **47**, 483-94.

9. Koenig, R. L., Ray, J. L., Maleki, S. J., Smeltzer, M. S. & Hurlburt, B. K. (2004). *Staphylococcus aureus* AgrA binding to the RNAIII-agr regulatory region. *J. Bacteriol.* **186**, 7549-7555.

10. Del Papa, M. F. & Perego, M. (2011). *Enterococcus faecalis* virulence regulator FsrA binding to target promoters. *J. Bacteriol.* **193**, 1527.

11. Risøen, P., Johnsborg, O., Diep, D., Hamoen, L., Venema, G. & Nes, I. (2001). Regulation of bacteriocin production in *Lactobacillus plantarum* depends on a conserved promoter arrangement with consensus binding sequence. *Mol. Genet. Genomics* **265**, 198-206.

12. Kraxenberger, T., Fried, L., Behr, S. & Jung, K. (2012). First insights into the unexplored two-component system YehU/YehT in *Escherichia coli*. *J. Bacteriol.* **194**, 4272-84.

13. Fried, L., Behr, S. & Jung, K. (2013). Identification of a target gene and activating stimulus for the YpdA/YpdB histidine kinase/response regulator system in *Escherichia coli*. *J. Bacteriol.* **195**, 807-815.

14. Behr, S., Fried, L. & Jung, K. (2014). Identification of a novel nutrient-sensing histidine kinase/response regulator network in *Escherichia coli*. *J. Bacteriol.*, JB. 01554-14.

15. Studier, F. W. & Moffatt, B. A. (1986). Use of bacteriophage T7 RNA polymerase to direct selective high-level expression of cloned genes. *J. Mol. Biol.* **189**, 113-130.

16. Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227**, 680-685.

17. Altschuh, D., Björkelund, H., Strandgård, J., Choulier, L., Malmqvist, M. & Andersson, K. (2012). Deciphering complex protein interaction kinetics using Interaction Map. *Biochem. Biophys. Res. Commun.* **428**, 74-79.

18. Barta, P., Björkelund, H. & Andersson, K. (2011). Circumventing the requirement of binding saturation for receptor quantification using interaction kinetic extrapolation. *Nucl. Med. Commun.* **32**, 863-867.

19. Leonard, P. G., Golemi-Kotra, D. & Stock, A. M. (2013). Phosphorylation-dependent conformational changes and domain rearrangements in *Staphylococcus aureus* VraR activation. *Proc. Natl. Acad. Sci. USA* **110**, 8525-8530.

20. Creager-Allen, R. L., Silversmith, R. E. & Bourret, R. B. (2013). A link between dimerization and autophosphorylation of the response regulator PhoB. *J. Biol. Chem.* **288**, 21755-21769.

21. Boudes, M., Sanchez, D., Graille, M., van Tilbeurgh, H., Durand, D. & Quevillon-Cheruel, S. (2014). Structural insights into the dimerization of the response regulator ComE from *Streptococcus pneumoniae*. *Nucleic Acids Res.*, gku110.

22. Vlahoviček, K., Kajan, L. & Pongor, S. (2003). DNA analysis servers: plot. it, bend. it, model. it and IS. *Nucleic Acids Res.* **31**, 3686-3687.

23. Narayanan, A., Kumar, S., Evrard, A. N., Paul, L. N. & Yernool, D. A. (2014). An asymmetric heterodomain interface stabilizes a response regulator–DNA complex. *Nat. Comm.* **5**.

24. Geisel, N. & Gerland, U. (2011). Physical limits on cooperative protein-DNA binding and the kinetics of combinatorial transcription regulation. *Biophys. J.* **101**, 1569-1579.

7 Histidine kinases and response regulators in networks

Kirsten Jung*, Luitpold Fried#, Stefan Behr#, and Ralf Heermann

Munich Center for Integrated Protein Science (CIPSM) at the Department of Microbiology, Ludwig-Maximilians-Universität München, 82152 Martinsried, Germany

# These authors contributed equally to this work

*To whom correspondence should be addressed:
Dr. Kirsten Jung
Ludwig-Maximilians-Universität München
Department Biologie I, Bereich Mikrobiologie
Großhaderner Str. 2-4
82152 Martinsried
Germany
Phone: +49-89-2180-74500
Fax: +49-89-2180-74520
E-mail: jung@lmu.de

Abstract

Two-component systems, composed of a histidine kinase (HK) and a response regulator (RR), are the major signal transduction devices in bacteria. Originally it was thought that these two components function as linear, phosphorylation-driven stimulus-response system. Here, we will review how accessory proteins are employed by HKs and RRs to mediate signal integration, scaffolding, interconnection and allosteric regulation, and how these two components are embedded in regulatory networks.

Full-text article:

http://www.ncbi.nlm.nih.gov/pubmed/22172627

http://www.sciencedirect.com/science/article/pii/S1369527411002086

Current Opinion in Microbiology, (2012), K. Jung, L. Fried, S. Behr and R. Heermann, Histidine kinases and response regulators in networks. Vol. 15, iss.2, p. 118-124, doi: 10.1016/j.mib.2011.11.009, Copyright (2011), Elsevier

8 Concluding discussion

8.1 The two-component system network of YehU/YehT and YpdA/YpdB at the onset of stationary phase – From overflow metabolism to carbon starvation

The LytS/LytTR two-component systems come into play when *Escherichia coli* cells pass exponential growth and face certain nutrient limitations. While YehU responds to the availability of peptides/amino acids (Chapter 2), YpdA perceives extracellular pyruvate concentrations above 250 µM (Chapter 3). Stimulus perception of YehU and YpdA, respectively, leads to signal transduction to their cognate response regulator proteins YehT and YpdB. In general signal transduction is achieved upon different protein phosphorylation steps. Whether this is also true for LytS/LytTR mediated signal transduction in *Escherichia coli* still remains elusive. Being activated YehT and YpdB, respectively, undergo conformational changes and bind to their corresponding target gene promoter regions (YehT to P_{yjiY} and YpdB to P_{yhjX}). This results in the expression of *yjiY* and *yhjX*, respectively. *yjiY* expression is furthermore controlled via cAMP/CRP. Known as catabolite repression the availability of more favorable carbon sources, like glucose, prevents *yjiY* expression. Low glucose concentrations in contrast activate the adenylate cyclase resulting in higher cyclic AMP levels. The formation of the cAMP/CRP complex mediates cellular reprogramming from anabolism to catabolism to bridge the perceived energy deficit (Green *et al.*, 2014). Hence cAMP/CRP facilitates *yjiY* expression upon stimulus perception, which was confirmed by the identification of a CRP consensus sequence in *yjiY* promoter DNA and a significantly reduced *yjiY* expression in a *cyaA* or *crp* deletion mutant (Chapter 2). Suprisingly expression of *yjiY* and *yhjX* is additionally coordinated. Induction of *yjiY* and subsequent production of the corresponding carbon starvation transport protein negatively influences *yhjX* expression. Vice versa expression of *yhjX* and subsequent production of the corresponding MFS-transport protein promotes *yjiY* expression (Chapter 4). It might be assumed, that the formation of a larger signaling unit mediates LytTR output through the interplay of both histidine kinases and both transport proteins (Figure 8.1). Such an interplay between sensory- and transport elements, so called trigger-transporter has already been described (Tetsch & Jung, 2009). Well characterized examples include the bacitracin resistance module BceS/BceAB of *Bacillus subtilis* (Dintner *et al.*, 2011) or the co-sensory system CadC/LysP in *E. coli* (Rauschmeier *et al.*, 2014), which provide an advantageous link of metabolic fluxes to transcriptional regulation (Västermark & Saier Jr, 2014). The domain architecture of YehU or YpdA, with at least five transmembrane helices but no obvious periplasmic ligand-binding domain, supports the idea of co-sensory functions for YjiY and YhjX (Chapter 4).

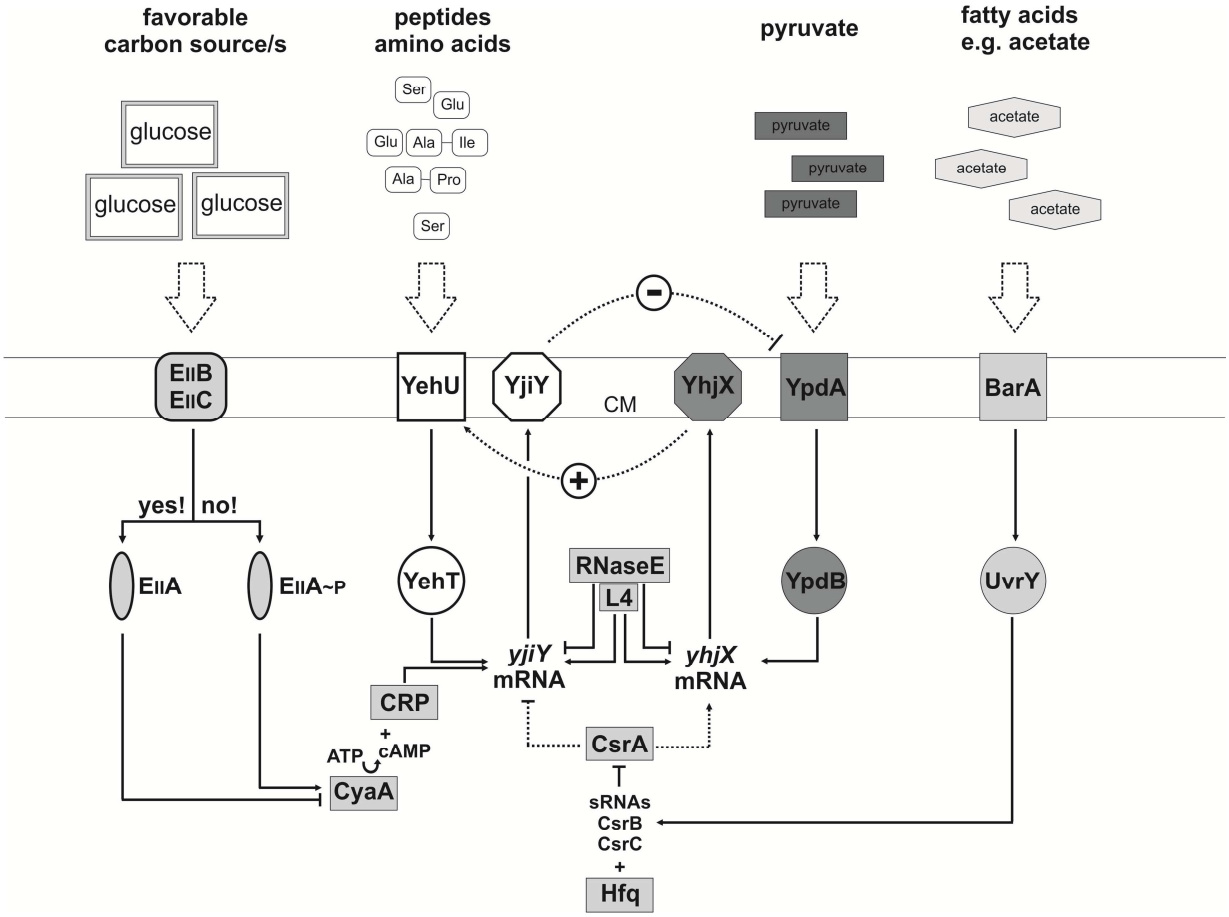

Figure 8.1 The YehU/YehT and YpdA/YpdB two-component systems as part of a large carbon sensing network. Based on current state of research this model summarizes the interplay between the two histidine kinase/response regulator systems YehU/YehT and YpdA/YpdB and their corresponding target proteins YjiY and YhjX. The influence of the PT (phosphotransferase) system as well as the effects of BarA/UvrY and the csr/RNase E/L4 regulation on *yjiY* and *yhjX* mRNA levels are depicted. Arrows indicate stimulating (↑) and repressing (⊥) effects. Dotted lines are solely based on in vivo evidence. Membrane proteins are integrated in the cytoplasmic membrane (CM). sRNAs, small RNAs; cAMP, cyclic AMP. For further detail see text.

When *E. coli* is grown in an amino acid-rich milieu, like LB medium both two-component systems YehU/YehT and YpdA/YpdB get activated at the transition from exponential to stationary growth phase. The exponential growth phase in nutrient rich environments is characterized by a term called overflow metabolism or the bacterial Crabtree effect. In this process consumption of a preferred carbon source is accompanied by secretion of several by-products to avoid bottlenecks (Paczia *et al.*, 2012). In particular pyruvate (Chapter 2) and acetate (Paczia et al., 2012) accumulate in the culture media and reach concentrations up to µM range. If the remaining carbon source is depleted, bacterial growth decelerates and excreted by-products (pyruvate and acetate) get retrieved in a process called scavenging (Peterson *et al.*, 2005). At that time expression of *yjiY* and *yhjX* is induced to mediate selectivity among the remaining nutrients. The interconnected response of YehU/YehT and YpdA/YpdB thereby links the

availability of amino acids and peptides to the abundance of the central metabolite pyruvate. Pyruvate is involved in several cellular processes, such as glycolysis, gluconeogenesis, fatty acids synthesis, amino acid synthesis and fermentation (Wolfe, 2005). As key molecule between respiration and fermentation and as a precursor for several macromolecules, its cellular concentration is tightly regulated (Vemuri *et al.*, 2006). Besides secretion (see above) pyruvate can also be specifically transported into the cell via at least two different import systems (Kreth *et al.*, 2013). Additionally the uptake of peptides or amino acids provides *E. coli* metabolism with further amounts of pyruvate from alanine, glycine or cysteine degradation. From this point of view (availability of peptides and amino acids) an additional uptake of pyruvate would offer *E. coli* no further benefit and, therefore *yhjX* expression is reduced. In contrast, the abundance of pyruvate, which strongly induces *yhjX* expression, might lead to precursor depletion or metabolic bottle necks, e.g. in the tricarboxylic acid cycle. In this situation parallel activation of *yjiY*, encoding a putative peptide transport protein, would be advantageous and could avoid this problem simply by refilling the precursor pool (Figure 8.2).

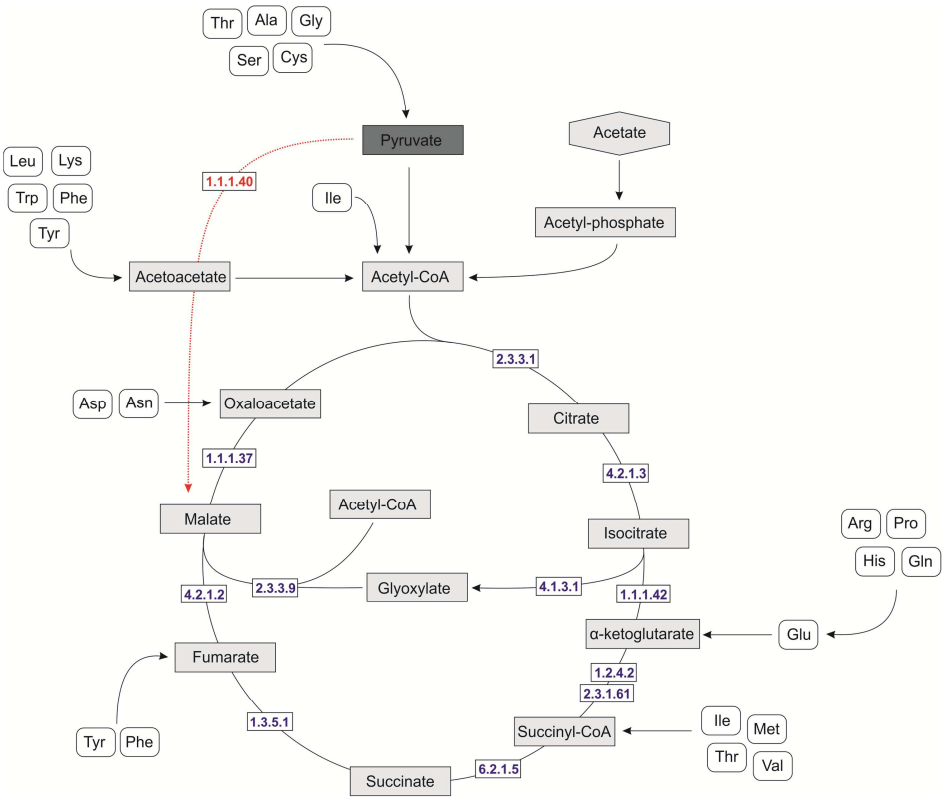

Figure. 8.2 Simplified scheme of the tricarboxylic acid cycle. Amino acids (depicted in the three-letter-code) from peptide degradation (can) act as precursors for different steps in the TCA cycle. In order to avoid energetically less favorable reactions (e.g. 1.1.1.40, the malate dehydrogenase, which converts pyruvate under the consumption of NADPH and CO_2 to generate malate) *E. coli* responds to the availability of pyruvate with an additional induction of *yjiY*, coding for putative peptide transporter. Given numbers represent KEGG PATHWAY annotations (EC numbers) for the corresponding enzymes (http://www.genome.jp/kegg/pathway.html).

Hence an optimal cellular carbon supply might be maintained in preparation for stationary phase and global reprogramming by sigma factor *rpoS* (Hengge-Aronis, 2002). The σ^S (RpoS) subunit of RNA-Polymerase is the master regulator of general stress response and regulates approximately 500 genes (nearly 10% of the genes in the *E. coli* genome) (Weber *et al.*, 2005) under several stress conditions (Lange & Hengge-Aronis, 1991). Furthermore microarray analyses indicated several metabolic adaptions in an *rpoS* mutant (Rahman *et al.*, 2006). These global consequences of switching between two entirely different life styles (exponential growth under carbon source excess *versus* dormant survival under nutrient limitations) need to be carefully controlled. The stimulation of YehU/YehT and YpdA/YpdB and the subsequent expression of *yjiY* and *yhjX* might counteract nutrient limitations at the interface to stationary growth phase and prepare cellular metabolism for upcoming adaptations. The regulatory role of another LytS/LytTR like two-component system at the transition from exponential to stationary growth phase was recently described. Based on quorum sensing the AgrC/AgrA two-component system from *Staphylococcus aureus* monitors the extracellular concentration of an autoinducing peptide (AIP) to regulate virulence gene expression (Novick & Geisinger, 2008). Nevertheless this signal transduction cascade can be overridden by the global transcriptional regulator CodY (Roux *et al.*, 2014). CodY directly senses intracellular concentrations of isoleucine and GTP levels (Pohl *et al.*, 2009) and hence seems to link cell density dependent virulence expression to individual nutrient availability.

This in turn directs the interest to the physiological role of YehU/YehT and YpdA/YpdB in *Escherichia coli*. The preferred natural habitat of enteric bacteria like *E. coli*, *Shigella flexneri* and *Salmonella typhimurium* can be found in the intestine of warm-blooded animals (Bearson *et al.*, 1997), e.g. the gastric juice. It is characterized by the abundance of nutrients like amino acids, such as alanine, proline or serine, and other carbon sources, e.g. pyruvate (Nagata *et al.*, 2003, Nagata *et al.*, 2007). Under these conditions both potential stimuli for YehU/YehT and YpdA/YpdB are present. Taking into account that *yjiY* and *yhjX* expression is furthermore tightly controlled and only occurs in a very distinct time slot this leads to speculations of a potential role in propagation or host colonization. In fact *yjiY* is expressed in vivo in avian pathogenic *E. coli* during the infection process in chicken liver and spleen (Tuntufye *et al.*, 2012). Both organs possess high amounts of peptides and amino acids (Brosnan, 2000, Mebius & Kraal, 2005). Another hypothesis deals with the idea of a unique adaption mechanism to colonize different host environments. In the human intestine uropathogenic *E. coli* (UPEC) is able to establish a commensal association in a microenvironment, which is characterized by an abundance of nutrients and a variety of bacterial competitors. In contrast UPEC transition to the urinary tract leads to a drastic reduction in the

availability of nutrients and bacterial competition and furthermore requires a great flexibility of carbon and energy metabolism (Alteri & Mobley, 2012).

8.2 Post-transcriptional mechanisms embed LytS/LytTR signal transduction into a complex cellular network of carbon utilization

In addition to the internal LytS/LytTR mediated carbon source hierarchy, several post-transcriptional mechanisms have been described how *yjiY* and *yhjX* mRNAs are regulated. This includes Csr (carbon storage regulator) one best studied global regulatory system. Within different organisms Csr has been shown to contribute to global regulatory processes like carbon control, the production of extracellular matrix compounds, cell motility, biofilm formation, quorum sensing and/or pathogenesis (Romeo *et al.*, 2012). In *Escherichia coli Csr* potentially regulates the expression of several hundred genes (Edwards *et al., 2011)* and is composed of five major components: CsrA is a homodimeric RNA-binding protein that regulates gene expression by affecting ribosome binding and/or mRNA stability (Babitzke & Romeo, 2007). Two non-coding small RNAs CsrB and CsrC (Liu et al., 1997, Weilbacher et al., 2003) antagonize CsrA function by sequestration. The BarA/UvrY two-component system in turn activates expression of *csrB* and *csrC*, while CsrD participates in RNase E mediated degradation of the small RNAs *(*Suzuki *et al.,* 2006). It is worth mentioning, that previous studies already showed CsrA dependent synthesis of the putative peptide transporter CstA (Dubey *et al.,* 2003). CstA shows a high amino acid sequence identity to YjiY. We observed that overexpression of *csrA* reduced *yjiY* expression, whereas the deletion of *csrA* resulted in constitutive *yjiY* expression (Chapter 4). Interestingly the effect of CsrA on *yhjX* expression appeared to be vice versa. Such a pleiotrophic regulation of CsrA was described e. g. for the central carbohydrate metabolism in *E. coli* (Sabnis *et al.,* 1995) and could provide a fine-tuned mechanism to integrate the intracellular metabolic state into nutrient selectivity. Nevertheless additional experiments will be necessary to proof binding of CsrA to the corresponding mRNAs of *yjiY* and/or *yhjX* to exclude indirect effects, e. g. due to changes in the copy number of only one transporter (Chapter 4).

Regulation of CsrA is subject to the small RNAs *csrB* and *csrC*, which are in turn under the transcriptional control of another two-component system BarA/UvrY (Figure 8.1). In *E. coli* BarA/UvrY is crucial for an efficient switch between glycolytic and gluconeogenic carbon sources (Pernestig *et al.*, 2003) and displays a pH-dependent activation (Mondragón *et al.*, 2006). BarA is physiologically stimulated by several short-chain aliphatic carboxylic fatty acids, such as acetate (Chavez *et al.*, 2010), which might suggest another functional interconnectivity. Utilization of pyruvate and acetate is closely linked (Tomar

et al., 2003) and both substrates highly accumulate upon overflow metabolism. Hence the BarA/UvrY two-component system might function to integrate the availability of fatty acids as another carbon source into LytS/LytTR mediated nutrient selectivity.

Besides the Csr system Hfq, a RNA-binding protein with chaperone activity (Moll *et al.*, 2003), seems to play an additional role for LytTR-mediated gene expression. An *hfq* deletion mutant exhibits a reduced cell division phenotype (Takada *et al.*, 1999) and furthermore led to reduced expression of *yjiY* and *yhjX,* respectively. Whether these observations are based on the absence of direct *hfq* dependent processes, like mRNA folding or effects on the stability of small RNAs, or simply originate from the reduced growth rate will be further investigated. Furthermore degradation of *yjiY* and *yhjX* mRNAs depends on the interplay of RNase E (Rne) and the ribosomal protein L4 (RplD). RNase E is a single-strand-specific endonuclease, which cleaves A/U rich sequences (Babitzke & Kushner, 1991) in a number of cellular mRNAs. The L4 protein is part of the 50S subunit of the ribosome and capable of transcriptional and post-transcriptional regulation (Freedman *et al.*, 1987). Singh et al. showed that binding of L4 to the catalytic domain of RNase E inhibits target specific cleavage. As a result increasing levels of *yjiY* and *yhjX* mRNAs were observed (Singh *et al.*, 2009).

In our model different aspects of nutrient selectivity are summarized (Figure 8.1). When *E. coli* grows on a preferred carbon source, like glucose or mannose, the PEP:sugar phosphotransferase system (PTS) inhibits the adenylate cyclase (CyaA) activity, which results in low intracellular cyclic AMP (cAMP) levels. The depletion of favorable carbon sources is accompanied by the raise of intracellular cAMP levels, which positively influences *yjiY* expression. In order to maintain an optimal cellular carbon supply, prior dispensable nutrients get associated. Thereby YehU/YehT and YpdA/YpdB coordinate the cellular response to peptides/amino acids and pyruvate by the formation of signaling unit together with YjiY and YhjX. In addition the BarA/UvrY TCS might integrate the availability of fatty acids (here: acetate) into the LytS/LytTR mediated response by triggering CsrA-dependent posttranscriptional regulation of *yjiY* and *yhjX* mRNAs. We suggest that this highly balanced regulatory network might be necessary to coordinate nutrient scavenging in order to readjust bacterial metabolism, e. g. in preparation for stationary phase or in order to explore new microenvironments.

8.3 The LytTR regulator YpdB and its molecular mechanism of DNA-binding

Recent studies have shown, that a variety of LytTR response regulators participate in virulence or virulence-associated mechanisms of many pathogenic bacteria (Table 8.1). Due to the structural properties of the LytTR DNA-binding domain an initially described consensus sequence seems to allow a greater variability than initially estimated (Del Papa & Perego, 2011). In contrast to that, the structural elements of the corresponding promoter DNA fragments are rather conserved. Nearly all LytTR regulators bind their DNA motifs in two direct repeats composed of 9 to 10 bps separated by 12 to 13 spacer bps (Table 8.1).

TABLE 8.1: Properties of LytTR response regulators

Regulator	Binding sequence (5'-3')	Spacer length (bps)	Regulated gene(s)	Reference
AgrA	(T/A)(A/C)(C/A)GTTN(A/G)(T/G)	12-13	*agr* locus, RNAIII	(Koenig *et al.*, 2004)
AlgR	CCGT(G/T)(C/G)(G/T)TC	-*	*fimU-pilVWXY1Y*, *hcnA*, *algD*	(Lizewski *et al.*, 2004)
BlpR	ATT(C/T)ANGANNT	10	*blp* operon	(de Saizieu *et al.*, 2000)
BrsR	ACCGTTTAG	12	*smu.150*, *smu.423*, *smu.1906*	(Xie *et al.*, 2010)
ComE	(A/T)CA(T/G)TT(C/G)(A/G)G	12	*comCDE*, *comAB*	(Ween *et al.*, 1999)
FsrA	(T/A)(T/C)A(A/G)GGA(A/G)	13	*fsrBDC*, *gelE-sprE*	(Del Papa & Perego, 2011)
PlnC, PlnD	TACGTTAAT	12	*pln* operon	(Risøen *et al.*, 2001)
VirR	CCCAGTT(A/C)T(T/G)CAC	8	*pfoA*, *ccp*, *virU*, *virT*, *vrr*	(Cheung & Rood, 2000)
YehT	ACC(G/A)CT(C/T)A	13	*yjiY*	(Chapter 2)
YpdB	GGCATTTCAT	11	*yhjX*	(Chapter 3)

* three binding sites

Interestingly nucleotides adjacent to the identified binding motifs seemed to influence promoter activity and hence gene expression (Chapter 2) (Chapter 3) (Del Papa & Perego, 2011). This led to the suggestion that the DNA-response regulator interaction and associated structural rearrangements on the DNA level might play a role in LytTR mediated gene expression as it already has been show e.g. for cAMP/CRP regulation (Hardwidge *et al.*, 2002).

Surface plasmon resonance spectroscopy measurements with the phosphorylated mimetic YpdB-D53E indicated the importance of LytTR RR phosphorylation for initial DNA binding (Chapter 6). Phosphorylation of response regulators is a common mechanism in many bacterial signal transduction systems (Jung *et al.*, 2012). It is often associated with conformational changes within the RR, which facilitate dimerization and subsequently induce gene expression. In accordance to that, recent structural studies on isolated receiver domain variants from the LytTR like RR ComE from *Streptococcus pneumoniae* demonstrated phosphorylation dependent monomer-to-dimer transition (Boudes *et al.*, 2014).

Besides the importance of phosphorylation further results from our SPR measurements suggest a novel allosteric two step binding mechanism. While binding of YpdB-D53E to *yhjX* promoter DNA is completely prevented upon substitution of motif M1, an interaction can still be observed when motif M2 is substituted (Chapter 6, Figures 6.2 and 6.3). This indicates that initial binding of a first RR molecule occurs on motif M1, before motif M2 can be occupied by a second RR molecule. Nevertheless earlier studies demonstrated that both motifs are essential for gene expression in vivo (Chapter 3). From our results we suggest, that the induction of conformational changes on DNA structure might facilitate binding to motif M2. Interestingly DNA analyses predicted an average curvature for motif M1 between 4 and 6 degree, whereas motif M2 curvature ranged from 8 and 11 degree (Vlahoviček *et al.*, 2003), which already indicates major structural differences between both motifs. In addition intermolecular interactions between both YpdB-D53E molecules might facilitate binding to motif M2, as it has been observed for KpdE recently (Narayanan *et al.*, 2014).

Finally target gene expression might be induced upon binding of the second YpdB-D53E molecule on motif M2, possibly by providing the required protein interface for RNA polymerase recruitment (Ptashne & Gann, 1997). For the LytTR domain of AgrA from *S. aureus* it was shown, that binding of two RR molecules induced strong DNA bending (Sidote et al., 2008), which could be an alternative mechanism to promote RNA polymerase binding. Interestingly the binding properties of YpdB-D53E to motif M2 revealed a high dissociation rate. This rapid promoter clearance could function as a mechanism to prevent excessive gene expression and might enable a dynamic pulse expression control in a more switch-like manner (Geisel & Gerland, 2011). This idea is supported by the fact that many LytTR regulated genes participate in tightly regulated processes, e. g. toxin production or pathogenicity.

8.4 Outlook

LytS/LytTR mediated signal transduction stills keeps a lot of secrets. The most fascinating questions deal with the following topics: What is the physiological role of these systems in the natural habitat of *Escherichia coli*? Is there any relationship to pathogenicity e.g. regarding host colonization in uropathogenic *E. coli*? Which compounds/stimuli are involved in signal perception? What are the ligands bound by the histidine kinases YehU and YpdA, and which stimuli are triggered via the transport proteins YjiY and YhjX? Which structural arrangements occur upon ligand binding and how do they affect the regulatory dynamics within the network? Which residues mediate contact sites for protein-protein or protein-DNA interaction? What are the structural properties of the accessory protein YehS and how is it functionally integrated into signal transduction?

In order to address these questions different approaches might be convenient. Based on well-established infection models different pathogenic organisms could be employed to identify effects of LytS/LytTR mediated gene expression on virulence or host pathogenicity. Deletion of corresponding signaling pathways could show how the carbon control network is integrated in bacterial virulence, as it was previously done for K^+ homeostasis via KpdD/KdpE (Freeman *et al.*, 2013). Furthermore bioinformatics could provide new insights into the physiology of LytS/LytTR signal transduction systems. The distribution of homologous systems (none, only one or both LytS/LytTR systems) in other enterobacteria and the correlation to their natural habitats/sites of infection (e.g. stomach, large intestine, small intestine) might lead to new findings. So it is conceivable that pyruvate and a certain composition of amino acids and peptides could also function as bacterial biomarker e.g. for colonization. To fully understand the molecular mechanisms of LytS/LytTR mediated signal transduction, it is furthermore necessary to unravel all compounds and signals. Purification of inducing supernatants followed by liquid chromatography and mass spectrometric analyses could help to identify the missing stimulus for YehU. Taking into account that the stimulus might be integrated via the transport proteins YjiY and YhjX makes this even more challenging. Combined expression studies in mutant strains lacking e.g. *ypdA*, *ypdB*, *yhjX* and *yjiY* as well as binding experiments (e.g. using isothermal titrations calorimetry or flow dialysis) with purified YehU and its potential ligand will be necessary to obtain proof (Gerharz *et al.*, 2003). Consistently the transport properties of YjiY and YhjX regarding their substrate specificity, their mode of transport (importer or exporter) and their energization have to be investigated (Jung *et al.*, 1998). Initial characterization of additional intracellular signals could be done by testing the influence of different mutant strains or media compositions on metabolic flux analyses (Holms, 1996, Valgepea *et al.*, 2010). Closely linked to this topic is next fascinating field of research: Which residues determine specificities

within the complex signaling unit? Which structural dynamics can be observed upon signal perception and how is signaling robustness ensured? To investigate residue specificities in vivo expression analyses as well as in vivo protein-protein interactions could be combined and verified with in vitro techniques like gel retardation experiments or co-immunoprecipitation (Gardner *et al.*, 2014). Hence substitutions of single amino acids, insertions or deletions of favorable regions could be constructed. To solve the structural dynamics of complex formation in vivo real time imaging of fluorescent hybrid protein fusions or fluorescence resonance energy transfer spectroscopy (Sommer, 2012) could be applied. The structural arrangement of individual complexes or temporal formations could also get visualized by cyro-electron tomography (Briegel *et al.*, 2012). Moreover solved protein structures will of course increase the knowledge e.g. regarding signal transduction, protein-protein interactions or ligand binding. This approach might also be very promising for the accessory protein YehS. Structural properties (e.g. binding of a certain co-factor) could lead to further insights into its accessory function and help to complete the picture of LytS/LytTR mediated signal transduction in *E. coli*.

8.5 References for Concluding Discussion

Alteri, C. J. & H. L. Mobley, (2012) *Escherichia coli* physiology and metabolism dictates adaptation to diverse host microenvironments. *Curr. Opin. Microbiol.* **15**: 3-9.

Babitzke, P. & S. R. Kushner, (1991) The Ams (altered mRNA stability) protein and ribonuclease E are encoded by the same structural gene of Escherichia coli. *Proc. Natl. Acad. Sci. U.S.A.* **88**: 1-5.

Babitzke, P. & T. Romeo, (2007) CsrB sRNA family: sequestration of RNA-binding regulatory proteins. *Curr. Opin. Microbiol.* **10**: 156-163.

Bearson, S., B. Bearson & J. W. Foster, (1997) Acid stress responses in enterobacteria. *FEMS Microbiol. Lett.* **147**: 173-180.

Boudes, M., D. Sanchez, M. Graille, H. van Tilbeurgh, D. Durand & S. Quevillon-Cheruel, (2014) Structural insights into the dimerization of the response regulator ComE from *Streptococcus pneumoniae*. *Nucleic Acids Res.*: gku110.

Briegel, A., X. Li, A. M. Bilwes, K. T. Hughes, G. J. Jensen & B. R. Crane, (2012) Bacterial chemoreceptor arrays are hexagonally packed trimers of receptor dimers networked by rings of kinase and coupling proteins. *Proc. Natl. Acad. Sci. U.S.A.* **109**: 3766-3771.

Brosnan, J. T., (2000) Glutamate, at the interface between amino acid and carbohydrate metabolism. *J. Nutr.* **130**: 988S-990S.

Chavez, R. G., A. F. Alvarez, T. Romeo & D. Georgellis, (2010) The physiological stimulus for the BarA sensor kinase. *J. Bacteriol.* **192**: 2009-2012.

Cheung, J. K. & J. I. Rood, (2000) The VirR response regulator from *Clostridium perfringens* binds independently to two imperfect direct repeats located upstream of the *pfoA* promoter. *J. Bacteriol.* **182**: 57.

de Saizieu, A., C. Gardes, N. Flint, C. Wagner, M. Kamber, T. J. Mitchell, W. Keck, K. E. Amrein & R. Lange, (2000) Microarray-based identification of a novel *Streptococcus pneumoniae* regulon controlled by an autoinduced peptide. *J. Bacteriol.* **182**: 4696-4703.

Del Papa, M. F. & M. Perego, (2011) *Enterococcus faecalis* virulence regulator FsrA binding to target promoters. *J. Bacteriol.* **193**: 1527.

Dintner, S., A. Staroń, E. Berchtold, T. Petri, T. Mascher & S. Gebhard, (2011) Coevolution of ABC transporters and two-component regulatory systems as resistance modules against antimicrobial peptides in Firmicutes bacteria. *J. Bacteriol.* **193**: 3851-3862.

Dubey, A. K., C. S. Baker, K. Suzuki, A. D. Jones, P. Pandit, T. Romeo & P. Babitzke, (2003) CsrA regulates translation of the *Escherichia coli* carbon starvation gene, *cstA*, by blocking ribosome access to the *cstA* transcript. *J. Bacteriol.* **185**: 4450-4460.

Edwards, A. N., L. M. Patterson-Fortin, C. A. Vakulskas, J. W. Mercante, K. Potrykus, D. Vinella, M. I. Camacho, J. A. Fields, S. A. Thompson, D. Georgellis, M. Cashel, P. Babitzke & T. Romeo, (2011) Circuitry linking the Csr and stringent response global regulatory systems. *Mol. Microbiol.* **80**: 1561-1580.

Freedman, L. P., J. M. Zengel, R. H. Archer & L. Lindahl, (1987) Autogenous control of the S10 ribosomal protein operon of *Escherichia coli*: genetic dissection of transcriptional and posttranscriptional regulation. *Proc. Natl. Acad. Sci. U.S.A.* **84**: 6516-6520.

Freeman, Z. N., S. Dorus & N. R. Waterfield, (2013) The KdpD/KdpE two-component system: integrating K+ homeostasis and virulence. *PLoS Pathog.* **9**: e1003201.

Gardner, S. G., K. D. Johns, R. Tanner & W. R. McCleary, (2014) The PhoU Protein from *Escherichia coli* Interacts with PhoR, PstB, and Metals To Form a Phosphate-Signaling Complex at the Membrane. *J. Bacteriol.* **196**: 1741-1752.

Geisel, N. & U. Gerland, (2011) Physical limits on cooperative protein-DNA binding and the kinetics of combinatorial transcription regulation. *Biophys. J.* **101**: 1569-1579.

Gerharz, T., S. Reinelt, S. Kaspar, L. Scapozza & M. Bott, (2003) Identification of basic amino acid residues important for citrate binding by the periplasmic receptor domain of the sensor kinase CitA. *Biochemistry* **42**: 5917-5924.

Green, J., M. R. Stapleton, L. J. Smith, P. J. Artymiuk, C. Kahramanoglou, D. M. Hunt & R. S. Buxton, (2014) Cyclic-AMP and bacterial cyclic-AMP receptor proteins revisited: adaptation for different ecological niches. *Curr. Opin. Microbiol.* **18**: 1-7.

Hardwidge, P. R., J. M. Zimmerman & L. J. Maher, 3rd, (2002) Charge neutralization and DNA bending by the *Escherichia coli* catabolite activator protein. *Nucleic Acids Res.* **30**: 1879-1885.

Hengge-Aronis, R., (2002) Signal transduction and regulatory mechanisms involved in control of the sigma(S) (RpoS) subunit of RNA polymerase. *Microbiol. Mol. Biol. Rev.* **66**: 373-395.

Holms, H., (1996) Flux analysis and control of the central metabolic pathways in *Escherichia coli*. *FEMS Microbiol. Rev.* **19**: 85-116.

Jung, H., S. Tebbe, R. Schmid & K. Jung, (1998) Unidirectional reconstitution and characterization of purified Na+/proline transporter of *Escherichia coli*. *Biochemistry* **37**: 11083-11088.

Jung, K., L. Fried, S. Behr & R. Heermann, (2012) Histidine kinases and response regulators in networks. *Curr. Opin. Microbiol.* **15**: 118-124.

Koenig, R. L., J. L. Ray, S. J. Maleki, M. S. Smeltzer & B. K. Hurlburt, (2004) *Staphylococcus aureus* AgrA binding to the RNAIII-agr regulatory region. *J. Bacteriol.* **186**: 7549-7555.

Kreth, J., J. W. Lengeler & K. Jahreis, (2013) Characterization of Pyruvate Uptake in *Escherichia coli* K-12. *PLoS One* **8**: e67125.

Lange, R. & R. Hengge-Aronis, (1991) Identification of a central regulator of stationary phase gene expression in *Escherichia coli*. *Mol. Microbiol.* **5**: 49-59.

Liu, M. Y., G. Gui, B. Wei, J. F. Preston, L. Oakford, Ü. Yüksel, D. P. Giedroc & T. Romeo, (1997) The RNA molecule CsrB binds to the global regulatory protein CsrA and antagonizes its activity in *Escherichia coli*. *JBC* **272**: 17502-17510.

Lizewski, S. E., J. R. Schurr, D. W. Jackson, A. Frisk, A. J. Carterson & M. J. Schurr, (2004) Identification of AlgR-regulated genes in *Pseudomonas aeruginosa* by use of microarray analysis. *J. Bacteriol.* **186**: 5672-5684.

Mebius, R. E. & G. Kraal, (2005) Structure and function of the spleen. *Nat. Rev. Immunol.* **5**: 606-616.

Moll, I., D. Leitsch, T. Steinhauser & U. Bläsi, (2003) RNA chaperone activity of the Sm-like Hfq protein. *EMBO Rep.* **4**: 284-289.

Mondragón, V., B. Franco, K. Jonas, K. Suzuki, T. Romeo, Ö. Melefors & D. Georgellis, (2006) pH-dependent activation of the BarA-UvrY two-component system in *Escherichia coli*. *J. Bacteriol.* **188**: 8303-8306.

Nagata, K., Y. Nagata, T. Sato, M. A. Fujino, K. Nakajima & T. Tamura, (2003) L-Serine, D- and L-proline and alanine as respiratory substrates of *Helicobacter pylori*: correlation between in vitro and in vivo amino acid levels. *Microbiology* **149**: 2023-2030.

Nagata, Y., T. Sato, N. Enomoto, Y. Ishii, K. Sasaki & T. Yamada, (2007) High concentrations of D-amino acids in human gastric juice. *Amino Acids* **32**: 137-140.

Narayanan, A., S. Kumar, A. N. Evrard, L. N. Paul & D. A. Yernool, (2014) An asymmetric heterodomain interface stabilizes a response regulator–DNA complex. *Nat. Comm.* **5**.

Novick, R. P. & E. Geisinger, (2008) Quorum sensing in staphylococci. *Ann. Rev. Genet.* **42**: 541-564.

Paczia, N., A. Nilgen, T. Lehmann, J. Gätgens, W. Wiechert & S. Noack, (2012) Extensive exometabolome analysis reveals extended overflow metabolism in various microorganisms. *Microb. Cell. Fact.* **11**: 122.

Pernestig, A. K., D. Georgellis, T. Romeo, K. Suzuki, H. Tomenius, S. Normark & O. Melefors, (2003) The *Escherichia coli* BarA-UvrY two-component system is needed for efficient switching between glycolytic and gluconeogenic carbon sources. *J. Bacteriol.* **185**: 843-853.

Peterson, C. N., M. J. Mandel & T. J. Silhavy, (2005) *Escherichia coli* starvation diets: essential nutrients weigh in distinctly. *J. Bacteriol.* **187**: 7549-7553.

Pohl, K., P. Francois, L. Stenz, F. Schlink, T. Geiger, S. Herbert, C. Goerke, J. Schrenzel & C. Wolz, (2009) CodY in *Staphylococcus aureus*: a regulatory link between metabolism and virulence gene expression. *J. Bacteriol.* **191**: 2953-2963.

Ptashne, M. & A. Gann, (1997) Transcriptional activation by recruitment. *Nature* **386**: 569-577.

Rahman, M., M. R. Hasan, T. Oba & K. Shimizu, (2006) Effect of *rpoS* gene knockout on the metabolism of *Escherichia coli* during exponential growth phase and early stationary phase based on gene expressions, enzyme activities and intracellular metabolite concentrations. *Biotechnol. Bioeng.* **94**: 585-595.

Rauschmeier, M., V. Schüppel, L. Tetsch & K. Jung, (2014) New insights into the interplay between the lysine transporter LysP and the pH sensor CadC in *Escherichia coli*. *J. Mol. Biol.* **426**: 215-229.

Risøen, P., O. Johnsborg, D. Diep, L. Hamoen, G. Venema & I. Nes, (2001) Regulation of bacteriocin production in *Lactobacillus plantarum* depends on a conserved promoter arrangement with consensus binding sequence. *Mol. Genet. Genomics* **265**: 198-206.

Romeo, T., C. A. Vakulskas & P. Babitzke, (2012) Post-transcriptional regulation on a global scale: form and function of Csr/Rsm systems. *Environ. Microbiol.* **1**: 1-12.

Roux, A., D. A. Todd, J. V. Velázquez, N. B. Cech & A. L. Sonenshein, (2014) CodY-Mediated Regulation of the *Staphylococcus aureus* Agr System Integrates Nutritional and Population Density Signals. *J. Bacteriol.*: JB. 00128-00113.

Sabnis, N. A., H. Yang & T. Romeo, (1995) Pleiotropic regulation of central carbohydrate metabolism in *Escherichia coli* via the gene *csrA*. *J. Biol. Chem.* **270**: 29096-29104.

Sidote, D. J., C. M. Barbieri, T. Wu & A. M. Stock, (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.

Singh, D., S. J. Chang, P. H. Lin, O. V. Averina, V. R. Kaberdin & S. Lin-Chao, (2009) Regulation of ribonuclease E activity by the L4 ribosomal protein of *Escherichia coli*. *Proc. Natl. Acad. Sci. U.S.A.* **106**: 864-869.

Sommer, E., (2012) Ph.D. thesis, Ruperto-Carola University of Heidelberg, Germany, In vivo study of the two-component signaling network in *Escherichia coli*.

Suzuki, K., P. Babitzke, S. R. Kushner & T. Romeo, (2006) Identification of a novel regulatory protein (CsrD) that targets the global regulatory RNAs CsrB and CsrC for degradation by RNase E. *Genes Dev.* **20**: 2605-2617.

Takada, A., M. Wachi & K. Nagai, (1999) Negative Regulatory Role of the *Escherichia coli hfq* Gene in Cell Division. *Biochem. Biophys. Res. Com.* **266**: 579-583.

Tetsch, L. & K. Jung, (2009) How are signals transduced across the cytoplasmic membrane? Transport proteins as transmitter of information. *Amino Acids* **37**: 467-477.

Tomar, A., M. Eiteman & E. Altman, (2003) The effect of acetate pathway mutations on the production of pyruvate in *Escherichia coli*. *Appl. Microbiol. Biotechnol.* **62**: 76-82.

Tuntufye, H. N., S. Lebeer, P. S. Gwakisa & B. M. Goddeeris, (2012) Identification of Avian pathogenic *Escherichia coli* genes that are induced in vivo during infection in chickens. *Appl. Environ. Microbiol.* **78**: 3343-3351.

Valgepea, K., K. Adamberg, R. Nahku, P.-J. Lahtvee, L. Arike & R. Vilu, (2010) Systems biology approach reveals that overflow metabolism of acetate in *Escherichia coli* is triggered by carbon catabolite repression of acetyl-CoA synthetase. *BMC Syst. Biol.* **4**: 166.

Västermark, Å. & M. H. Saier Jr, (2014) The involvement of transport proteins in transcriptional and metabolic regulation. *Curr. Opin. Microbiol.* **18**: 8-15.

Vemuri, G. N., E. Altman, D. P. Sangurdekar, A. B. Khodursky & M. A. Eiteman, (2006) Overflow metabolism in *Escherichia coli* during steady-state growth: transcriptional regulation and effect of the redox ratio. *Appl. Environ. Microbiol.* **72**: 3653-3661.

Vlahoviček, K., L. Kajan & S. Pongor, (2003) DNA analysis servers: plot. it, bend. it, model. it and IS. *Nucleic Acids Res.* **31**: 3686-3687.

Weber, H., T. Polen, J. Heuveling, V. F. Wendisch & R. Hengge, (2005) Genome-wide analysis of the general stress response network in *Escherichia coli*: σS-dependent genes, promoters, and sigma factor selectivity. *J. Bacteriol.* **187**: 1591-1603.

Ween, O., P. Gaustad & L. S. Havarstein, (1999) Identification of DNA binding sites for ComE, a key regulator of natural competence in *Streptococcus pneumoniae*. *Mol. Microbiol.* **33**: 817-827.

Weilbacher, T., K. Suzuki, A. K. Dubey, X. Wang, S. Gudapaty, I. Morozov, C. S. Baker, D. Georgellis, P. Babitzke & T. Romeo, (2003) A novel sRNA component of the carbon storage regulatory system of *Escherichia coli*. *Mol. Microbiol.* **48**: 657-670.

Wolfe, A. J., (2005) The acetate switch. *Microbiol. Mol. Biol. Rev.* **69**: 12-50.

Xie, Z., T. Okinaga, G. Niu, F. Qi & J. Merritt, (2010) Identification of a novel bacteriocin regulatory system in *Streptococcus mutans*. *Mol. Microbiol.* **78**: 1431-1447.

9. Supplemental Material – Chapter 1

Supplemental Material:

First insights into the unexplored two-component system YehU/YehT in *Escherichia coli*

Tobias Kraxenberger[1,2#], Luitpold Fried[1#], Stefan Behr[1], and Kirsten Jung[1*]

[1]Munich Center for Integrated Protein Science (CIPSM) at the Department of Microbiology,

Ludwig-Maximilians-Universität München, 82152 Martinsried, Germany

[2]current address:

AMSilk GmbH

Am Klopferspitz 19

82152 Planegg/Martinsried

[#] These authors contributed equally to this work

Running title: Response regulator YehT in *Escherichia coli*

*To whom correspondence should be addressed:
Dr. Kirsten Jung
Ludwig-Maximilians-Universität München
Department Biologie I, Bereich Mikrobiologie
Großhaderner Str. 2-4
82152 Martinsried
Germany
Phone: +49-89-2180-74500
Fax: +49-89-2180-74520
E-mail: jung@lmu.de

TABLE S1. Oligonucleotides used in this study

Oligonucleotide	Oligonucleotide Sequence (5´-3´)	Description
Plasmid or strain construction		
yehT XbaI anti	TTTTCTAGATTACAGGCCAATCGCCTCTTT	pBAD24-*yehT*
yehT N-6His EcoRI sense	TTTGAATTCATGCATCATCATCATCATCATAGCAGCGGCCATATCGAAGG TCGTCATATGATTAAAGTCTTAATTGTC	pBAD24-*yehT*
D54E sense	CCGGATGTGCTGTTTCTCGAGATCCAGATGCCGCGCATC	pBAD24-*yehT*-D54E
D54E anti	GATGCGCGGCATCTGGATCTCGAGAAACAGCACATCCGG	pBAD24-*yehT*-D54E
D54N sense	GTGCTGTTTCTCAATATCCAGATGC	pBAD24-*yehT*-D54N
D54N anti	GCATCTGGATATTGAGAAACAGCAC	pBAD24-*yehT*-D54N
kdpE sense	TTTCGCCATATGACAAACGTTCTGATTGT	pBAD24-*kdpE*
kdpE antisense	CTCTCTAGATCAAAGCATAAACCGATAGC	pBAD24-*kdpE*
yjiY EcoRI sense	ATACCGGAATTCATGGATACTAAAAAGATATTCAAGCACA	pBAD24-*yjiY*
yjiY C-6His XbaI anti	TTTTCTAGATTAATGATGATGATGATGATGGCCGCCGCTGCTACGACCTT CGATCATATGGTGGTGCGAAGAGATCTTCACGCCGCCTT	pBAD24-*yjiY*
yehU EcoRI sense	TCGGAATTCATGTACGATTTTAATCTGGTG	pBAD24-*yehU*, pBAD24-*yehUT*
yehU C-6his XbaI	TTTTCTAGATTAATGATGATGATGATGATGGCCGCCGCTACGACCTT CGATCATATGTGCCTCGTCC	pBAD24-*yehU*
H382Q sense	GGTGAATCCCCAATTTTTGTTTAAT	pBAD24-*yehU* H382Q
H382Q anti	ATTAAACAAAAATTGGGGATTCACC	pBAD24-*yehU* H382Q
pBAD24 anti	CAAATTCTGTTTTATCAGACCGCTTCTGCG	pBAD24 sequencing
pBAD24 sense	TCGCAACTCTCTACTGTTTCTCCATA	pBAD24 sequencing
rev24	TTCACACAGGAAACAGCTATGACC	pUC19 sequencing, labeling EMSA
uni24	ACGACGTTGTAAAACGACG	pUC19 sequencing
up yjiY 300bp BamHI sense	AATCCGGATCCCGCCGAGTGAATTTTATTCACACTCTGAA	pBBR *yjiY-lux*
up yjiY EcoRI anti	ATACCGGAATTCAGTAAAACCTGGCATGTATTGATTA	pBBR *yjiY-lux*
up yjiY Del CRP BS sense	TAACGCGTGTCGCGTCCGACACGCGTTATCGTCACTTAAACGACGCCTT	pBBR *yjiY*-sub-CRP-BS-*lux*
up yjiY Del CRP BS anti	TAAATTATTCACAATATAACGCGTGTCGCGTCCGACACGCGTTA	pBBR *yjiY*-sub-CRP-BS-*lux*
UP-YehU	ACGCAGGCAATGTATGTTACGCGTTTTAAAGGGAAGTGTGGTTTGCGGG TAATTAACCCTCACTAAAGGGCG	*E. coli* MG2 construction
LOW-YehT	GCATGAGGCCTTCAGGTGTTGATGAGGCAAAAAGCCATTTTAGCAGTCTT TAATACGACTCACTATAGGGCTC	*E. coli* MG2 construction
RED-Kan anti	CGAGACTAGTGAGACGTGCTAC	control primer
RED-Kan sense	TATCAGGACATAGCGTTGGCTACC	control primer
yehT - 50	CGTTACTTAGCATGAGGCCTT	control primer
yehT + 50	TGTGAGCCTGATAGTTACACC	control primer
yehT-rpsL-neo-DOWN	CAAGCATCCCCACCATTTCCAGACCACTGATGCGCGGCATCTGGATATTC AGAAGAACTCGTCAAGAAGG	*E. coli* MG3 construction
yehT-rpsL-neo-up	GAAGGGATCGGCGCGGTGCATAAACTGCGCCCGGATGTGCTGTTTCTCG GCCTGGTGATGATGGCGGGAT	*E. coli* MG3 construction
yehU-rpsL-neo-DOWN	GGGATTCACCTGGGCGTGAAGCAGTTTGATCTCTGACTGGGTGAGCATT CAGAAGAACTCGTCAAGAAGG	*E. coli* MG6 construction
yehU-rpsL-neo-UP	TAAAAATACACTTCGGCTTTTTCTGCCACTGCGCTGAAAGTGGCAGCCGG CCTGGTGATGATGGCGGGAT	*E. coli* MG6 construction
YehTU test anti	GAATAAACAGATGTGTGGTGAGTGT	control primer
YehTU test sense	AAACCCTCTTCGTCTTCTTTACGT	control primer
yehU - 50	GCTCCTGCAAAAATACACGCA	control primer

yehU + 50	CTGCAAGAGTTCAAAGAAAGT	control primer
yehU sense	ATGTACGATTTTAATCTGGTG	control primer
yehU anti	TCATGCCTCGTCCCTCCATGG	control primer
up yjiY	ACCTAGAACGGCTTCGGCCAACTATTAATCAATACATGCCAGGTTTTACT AATTAACCCTCACTAAAGGGCG	*E. coli* MG10 construction
down yjiY	TAGTTCACTCTGATAAGAACAAAGCCCCGCCGAAGCGGGGCTAAACACG GTAATACGACTCACTATAGGGCTC	*E. coli* MG10 construction
yjiY -200	CGTTCGCGGAAGAATTCTTCATA	control primer
up cyaA	GATGTTGGCGGAATCACAGTCATGACGGGTAGCAAATCAGGCGATACGT CAATTAACCCTCACTAAAGGGCG	*E. coli* MG11 construction
down cyaA	GTTTCCGCTAAGATTGCATGCCGGATAAGCCTCGCTTTCCGGCACGTTCA TAATACGACTCACTATAGGGCTC	*E. coli* MG11 construction
cya + 200	GCGCATCTTTCTTTACGGTCAAT	control primer
cya - 200	AGGAGCCGCTGCACCAGGTAT	control primer
up CRP	AGAAAGCTTATAACAGAGGATAACCGCGCATGGTGCTTGGCAAACCGCA AAATTAACCCTCACTAAAGGGCG	*E. coli* MG12 construction
down CRP	GCTACCAGGTAACGCGCCACTCCGACGGGATTAACGAGTGCCGTAAACG ATAATACGACTCACTATAGGGCTC	*E. coli* MG12 construction
CRP + 200	GACACAAAGCGAAAGCTATGCTAAA	control primer
CRP - 200	GTTCCTGCCTGTTGCAATATTGCG	control primer

Northern Blot DNA probes		
alsA sense	ATGGCCACGCCATATATATCG	*alsA* probe
alsA anti	CCAGGGGTGAACGTGGAGAGA	*alsA* probe
arpB sense	ATGAGTCAAAACGATATCATT	*arpB_1 & arpB_2* probe
arpB anti	CTACCATTGAGATTTACTGTT	*arpB_1 & arpB_2* probe
cspI sense	ATGTCTAACAAAATGACTGGT	*cspI* probe
cspI anti	TCAAAGCGCCACTACATGAAC	*cspI* probe
cysB sense	ATGAAATTACAACAACTTCGC	*cysB* probe
cysB anti	TTATTTTTCCGGCAGTTTTAT	*cysB* probe
evgA anti	TTAGCCGATTTTGTTACGTTG	*evgAS* probe
evgA sense	ATGAACGCAATAATTATTGAT	*evgAS* probe
fimB sense	ATGAAGAATAAGGCTGATAAC	*fimB* probe
fimB anti	CTATAAAACAGCGTGACGCTG	*fimB* probe
fimE sense	GTGAGTAAACGTCGTTATCTT	*fimE* probe
fimE anti	TCAAACCTCTTCTCTTTTTAA	*fimE* probe
gadB sense	ATGGATAAGAAGCAAGTAACG	*gadB* probe
gadB anti	GGCGCAGGAATTCATAGTACT	*gadB* probe
ivbL sense	ATGACTACTTCCATGCTCAAC	*ivbL* probe
ivbL anti	CTACGGCGCATTGCCGACGAC	*ivbL* probe
kdsB sense	ATGAGTTTTGTGGTCATTATT	*kdsB* probe
kdsB anti	TTAGCGCATTTCAGCGCGAAC	*kdsB* probe
nlpA sense	ATGAAACTGACAACACATCAT	*nlpA* probe
nlpA anti	TTACCAGCCAGGCACCGCGCC	*nlpA* probe
nupA sense	ATGAAAAAAACATTACTGGCA	*nupA* probe
nupA anti	TCAGAAGTTGTAACCTACTAC	*nupA* probe
ompT sense	ATGCGGGCGAAACTTCTGGGA	*ompT* probe
ompT anti	TTAAAATGTGTACTTAAGACC	*ompT* probe
rpoD sense	ATGGAGCAAAACCCGCAGTCAC	*rpoD* probe
rpoD anti	AATCGTCCAGGAAGCTACGCAGC	*rpoD* probe
yadC sense	ATGAAGACTATCTTCAGGTAC	*yadC* probe
yadC anti	GTACGCCAACGCCTTTGGCGG	*yadC* probe
yahN sense	ATGATGCAGTTAGTTCACTTA	*yahN* probe
yahN anti	TCACCGCTGCGTCACCCCTTC	*yahN* probe
ybbC sense	ATGAAATATAGTTCAATATTT	*ybbC* probe
ybbC anti	TTATTTACTATGTAGGAAATT	*ybbC* probe

ybcK sense	ATGAAAAAAGCCATAGCATAT	*ybcK* probe
ybcK anti	TCATCGGACAAACATAATAGC	*ybcK* probe
yebK sense	ATGAATATGCTGGAAAAAATC	*yebK* probe
yebK anti	TTAGCGATCGTCACTTAAATT	*yebK* probe
yeiL sense	ATGAGTGAATCCGCGTTTAAG	*yeiL* probe
yeiL anti	TTACTGCATCATCCCGGAGAA	*yeiL* probe
yfcV sense	ATGAGTAAGTTTGTTAAAACA	*yfcV* probe
yfcV anti	TTACAGGTAAGTAATCTGGAA	*yfcV* probe
yfiL sense	ATGATGAAAAAGTTTATCGCC	*yfiL* probe
yfiL anti	TTAATTTAATCGTATTGTGCT	*yfiL* probe
yhjX sense	ATGACACCTTCAAATTATCAG	*yhjX* probe
yhjX anti	CAAAGAACTCACTGACCAGTG	*yhjX* probe
yibG sense	ATGAAAGCATGCTTGTTACTA	*yibG* probe
yibG anti	TTACGGATTCTCCTTATTCTT	*yibG* probe
yigF sense	ATGAGTAAGGAATATATGAAC	*yigF* probe
yigF anti	TCAAAGGCTCCAGGTATTTAA	*yigF* probe
yjiY sense	ATGGATACTAAAAAGATATTC	*yjiY* probe
yjiY anti	TGATGAACAGGAACGGGAACA	*yjiY* probe
ylbH sense	ATGAGCGGAAAACCAGCGGCG	*ylbH* probe
ylbH anti	TGCTGCGGTCATATACCGCCA	*ylbH* probe
yobA sense	ATGGCTTCAACTGCACGCTCC	*yobA-yebZY* probe
yobA anti	TTATTTCACGCTAAAGGTGTA	*yobA-yebZY* probe
ypjB sense	ATGGAATCACGTAATTCATAT	*ypjB* probe
ypjB anti	TCATTGAAAACTGCTTTTAGA	*ypjB* probe
ytfI sense	ATGTTACCCAGGATCAGACAC	*ytfI* probe
ytfI anti	TTAAGTCTCTGAGTTCTTACG	*ytfI* probe

5´RACE

5PR RNA Adapter*	GAUAUGCGCGAAUUCCUGUAGAACGAACACUAGAAGAAA	(1)
5´R Adapter Primer III	TGTAGAACGAACACTAGAAGAA	5´RACE Adapter
5PR Primer II	GCGCGAATTCCTGTAGA	5´RACE
cspI-c-anti	CCGTTCTCAATACCAAATTCAACT	*cspI* 5´RACE
cspI-cII-anti	CACATCTTTGCTGCCATCTT	*cspI* 5´RACE
cspI-c-III	CCTTTTTCAGGGTTAAACCATT	*cspI* 5´RACE
yhjX-c-anti	AAACAGGCTCCAGGTATAAACC	*yhjX* 5´RACE
yhjX-c-anti II	CGCAACAGAAGACGAAATTG	*yhjX* 5´RACE
yjiY c anti II	GTTCAGACCGTCGTTGTTAATAA	*yjiY* 5´RACE
yjiY-c-anti	CCGACGTAATGCAACTACCG	*yjiY* 5´RACE
yjiY-cII-anti	GTTCAGACCGTCGTTGTTAATAA	*yjiY* 5´RACE
ypjB-c-anti	TCTATGGGGTGTTCCTTT	*ypjB* 5´RACE
ypjB-c-anti II	GCTCTTGGTAAAAACTTATAGCAAC	*ypjB* 5´RACE

EMSA/footprint

6-FAM uni24	[6-FAM]ACGACGTTGTAAAACGACGGCCAG	EMSA labeling DNA-fragments
cspI 1 anti	TTTTTTGGATCCTGAGAAATGGACAAACAC	pUC19 $P_{cspI\ -150/+250}$
cspI 4 sense	TTTTTTGAATTCGCCAAAATTCCTGAAATC	pUC19 $P_{cspI\ -150/+250}$
yhjX 5PR 1 anti	TTTTTTGGATCCTAAACAGGCTCCAGGTATAA	pUC19 $P_{yhjX\ -173/+137}$
yhjX 5PR 3 sense	TTTTTTGAATTCTAACAATAGTTGTGGCGATAGTGG	pUC19 $P_{yhjX\ -173/+137}$
yjiY YehT bs	TTTTTTGAATTCCCTTTGCCGCTCAACCGCAAAACTGACCGCTTACATCC CTAAAATAACCACTCAGTTAGGATCCTTTTTT	pUC19 $P_{yjiY\ P}$
yjiY YehT bs anti	AAAAAAGGATCCTAACTGAGTGGTTATTTTAGGGATGTAAGCGGTCAGTT TTGCGGTTGAGCGGCAAAGGGAATTCAAAAAA	pUC19 $P_{yjiY\ P}$
yjiY YehT bs mut 1	TTTTTTGAATTCCCTTTGAATAGACACCGCAAAACTGACCGCTTACATCCC TAAAATAACCACTCAGTTAGGATCCTTTTTT	pUC19 $P_{yjiY\ MM3}$

yjiY YehT bs mut 1 anti	AAAAAAGGATCCTAACTGAGTGGTTATTTTAGGGATGTAAGCGGTCAGTT TTGCGGTGTCTATTCAAAGGGAATTCAAAAAA	pUC19 $P_{yjiY\ MM3}$
yjiY YehT bs mut 2	TTTTTTGAATTCCCTTTGAATAGACACCGCAAAACTGAAATAGGCCATCCC TAAAATAACCACTCAGTTAGGATCCTTTTTT	pUC19 $P_{yjiY\ MM23}$
yjiY YehT bs mut 2 anti	AAAAAAGGATCCTAACTGAGTGGTTATTTTAGGGATGGCCTATTTCAGTTT TGCGGTGTCTATTCAAAGGGAATTCAAAAAA	pUC19 $P_{yjiY\ MM23}$
yjiY YehT bs mut 3	TTTTTTGAATTCCCTTTGAATAGACACCGCAAAACTGAAATAGGCCATCCC TAAAATAAAAGAGACGTTAGGATCCTTTTTT	pUC19 $P_{yjiY\ MM123}$
yjiY YehT bs mut 3 anti	AAAAAAGGATCCTAACGTCTCTTTTATTTTAGGGATGGCCTATTTCAGTTT TGCGGTGTCTATTCAAAGGGAATTCAAAAAA	pUC19 $P_{yjiY\ MM123}$
yjiY YehT bs spacer mut	TTTTTTGAATTCCCTTTGCCGCTCACAATACCCCAGTCCCGCTTAACGAAA GCCCCGCCCCACTCAGTTAGGATCCTTTTTT	pUC19 $P_{yjiY\ MS}$
yjiY YehT bs spacer mut anti	AAAAAAGGATCCTAACTGAGTGGGGCGGGGCTTTCGTTAAGCGGGACTG GGGTATTGTGAGCGGCAAAGGGAATTCAAAAAA	pUC19 $P_{yjiY\ MS}$
yjiY-5P-1 anti	TTTTTTGGATCCAGTAAAACCTGGCATGTA	pUC19 $P_{yjiY\ -212/+88}$, pUC19 $P_{yjiY\ -12/+88}$, pRS415 $P_{yjiY\ -212/+88}$ + derivates
yjiY-5P-1 sense	TTTTTTGAATTCCAACATCACTACAGGATAG	pUC19 $P_{yjiY\ -12/+88}$
yjiY-5P-2 anti	TTTTTTGGATCCCCAGAGTTACGCGCGCGT	pUC19 $P_{yjiY\ -112/-13}$
yjiY-5P-2 sense	TTTTTTGAATTCCTTAAACGACGCCTTTGCCGC	pUC19 $P_{yjiY\ -112/-13}$
yjiY-5P-3 anti	TTTTTTGGATCCTGACGATAAATATGTGAT	pUC19 $P_{yjiY\ -212/-113}$
yjiY-5P-3 sense	TTTTTTGAATTCCGCCGAGTGAATTTTATTCA	pUC19 $P_{yjiY\ -212/+88}$, pUC19 $P_{yjiY\ -212/-113}$ pRS415 $P_{yjiY\ -212/+88}$ + derivates
ypjB 5PR 1 anti	TTTTTTGGATCCGAACTAACTAACTCGTGATTA	pUC19 $P_{ypjB\ -170/+130}$
ypjB 5PR 3 sense	TTTTTTGAATTCATTTAACGCTAGCGCAGTTTT	pUC19 $P_{ypjB\ -170/+130}$

In vivo reporter

2STPCR_ms_sense	CAATACCCCAGTCCCGCTTAACGAAAGCCCCGCC	pRS415 $P_{yjiY\ MS}$
2STPCR_ms_antis	GGCGGGGCTTTCGTTAAGCGGGACTGGGGTATTG	pRS415 $P_{yjiY\ MS}$
2STPCR_m123_sense	AATAGACACCGCAAAACTGAAATAGGCCATCCCTAAAATAAAAGAGAC	pRS415 $P_{yjiY\ M123}$
2STPCR_m123_anti	GTCTCTTTTATTTTAGGGATGGCCTATTTCAGTTTTGCGGTGTCTATT	pRS415 $P_{yjiY\ M123}$
2STPCR_m23_sense	AATAGACACCGCAAAACTGAAATAGGC	pRS415 $P_{yjiY\ M23}$
2STPCR_m23_anti	GCCTATTTCAGTTTTGCGGTGTCTATT	pRS415 $P_{yjiY\ M23}$
2STPCR_m3_sense	AATAGACACCGCAAAACTGAC	pRS415 $P_{yjiY\ M3}$
2STPCR_m3_anti	GTCAGTTTTGCGGTGTCTATT	pRS415 $P_{yjiY\ M3}$
yjiY spacer -1 sense	CATCCCGAAAATAACCACTCAG	pRS415 $P_{yjiY\ SC1}$
yjiY spacer -1 antisense	CTGAGTGGTTATTTTCGGGATG	pRS415 $P_{yjiY\ SC1}$
yjiY spacer -3 sense	CATCCAGCAAATAACCACTCAG	pRS415 $P_{yjiY\ SC3}$
yjiY spacer -3 antisense	CTGAGTGGTTATTTGCTGGATG	pRS415 $P_{yjiY\ SC3}$
yjiY spacer -7 sense	CATAAAGCCCATAACCACTCAG	pRS415 $P_{yjiY\ SC7}$
yjiY spacer -7 antisense	CTGAGTGGTTATGGGCTTTATG	pRS415 $P_{yjiY\ SC7}$
yjiY spacer -13 sense	ACGAAAGCCCCGCACCACTCAGTTATT	pRS415 $P_{yjiY\ SC13}$
yjiY spacer -13 antisense	AATAACTGAGTGGTGCGGGGCTTTCGT	pRS415 $P_{yjiY\ SC13}$

* RNA-Oligonucleotide

TABLE S2. Genes most affected by the overexpression of *yehT* or *kdpE.*

gene[a]	b-number[a]	rF (YehT)[b]	rF (KdpE)[b]	log2 Ratio[b]	p[c]	function[a]	transcriptional regulation[d]
yjiY	b4354	9,200	50	7.6	$\leq 10^{-3}$	predicted inner membrane protein	YehT ↑
evgA	b2369	3,000	70	5.4	$\leq 10^{-3}$	DNA-binding transcriptional activator EvgA	KdpE ↓
yobA	b1841	3,200	170	4.2	$\leq 10^{-3}$	hypothetical protein	KdpE ↓
nlpA	b3661	1,300	70	4.2	0.003	cytoplasmic membrane lipoprotein-28	KdpE ↓
evgS	b2370	700	40	4.2	$\leq 10^{-3}$	hybrid HK in two-component regulatory system with EvgA	KdpE ↓
yebZ	b1840	2,100	150	3.8	$\leq 10^{-3}$	putative resistance protein	KdpE ↓
nupA	b0411	1,700	140	3.6	0.001	nucleoside channel	KdpE ↓
ivbL	b3672	3,700	330	3.5	$\leq 10^{-3}$	*ilvB* operon leader peptide	KdpE ↓
ompT	b0565	8,200	770	3.5	$\leq 10^{-3}$	outer membrane protease	KdpE ↓
yahN	b0328	530	60	3.3	$\leq 10^{-3}$	putative cytochrome subunit of dehydrogenase	KdpE ↓
yebK	b1853	1,880	210	3.2	$\leq 10^{-3}$	DNA-binding transcriptional regulator HexR	KdpE ↓
cysB	b1275	1,890	230	3.0	$\leq 10^{-3}$	transcriptional regulator CysB	KdpE ↓
kdsB	b0918	2,390	300	3.0	$\leq 10^{-3}$	3-deoxy-manno-octulosonate cytidylyltransferase	KdpE ↓
cspI	b1552	300	2,480	-3.0	0.01	cold shock-like protein CspI	YehT ↓
fimB	b4312	950	7,810	-3.1	0.001	tyrosine recombinase/inversion of on/off regulator of *fimA*	KdpE ↑
yadC	b0135	50	510	-3.2	$\leq 10^{-3}$	predicted fimbrial-like adhesin protein	KdpE ↑
yigF	b3817	50	510	-3.3	$\leq 10^{-3}$	conserved inner membrane protein	KdpE ↑
gadB	b1493	750	7,340	-3.5	$\leq 10^{-3}$	glutamate decarboxylase B. PLP-dependent	KdpE ↑
arpB_2	b1721	40	420	-3.9	0.001	hypothetical protein	KdpE ↑
ybbC	b0498	40	420	-4.2	0.001	predicted protein	KdpE ↑
alsA	b4087	180	3,110	-4.4	$\leq 10^{-3}$	fused D-allose transporter subunits of ABC superfamily: ATP-binding components	KdpE ↑
yibG	b3596	30	530	-4.5	$\leq 10^{-3}$	hypothetical protein	KdpE ↑
yeiL	b2163	30	590	-4.5	$\leq 10^{-3}$	DNA-binding transcriptional activator	KdpE ↑
yfcV	b2339	50	1,020	-4.6	0.003	predicted fimbrial-like adhesin protein	KdpE ↑
ytfI	b4215	10	180	-4.8	0.008	hypothetical protein	KdpE ↑
yfiL	b2602	30	830	-5.0	0.001	hypothetical protein	KdpE ↑
ylbH	b0499	180	5,480	-5.1	0.001	protein in rhs loci	KdpE ↑
ybcK	b0544	70	2,540	-5.2	$\leq 10^{-3}$	DLP12 prophage; predicted recombinase	KdpE ↑
fimE	b4313	10	400	-5.3	0.003	tyrosine recombinase	KdpE ↑
yhjX	b3547	80	3,120	-5.3	$\leq 10^{-3}$	uncharacterized member of the major facilitator superfamily (MFS) of transporters	YehT ↓
arpB_1	b1720	200	7,980	-6.9	0	hypothetical protein	KdpE ↑
ypjB	b2649	20	1,790	-8.3	$\leq 10^{-3}$	hypothetical protein	YehT ↓

[a] Gene names/b-numbers and gene product function are adopted from http://www.ecocyc.org (3) and the Affymetrix Expression Analysis Sequence Information Database (2).

[b] Log_2 ratio of transcript levels for the *yehT*- and *kdpE*-overexpression strains. Log_2 was calculated from the ratio of the mean fluorescence intensity of the respective transcript in the *yehT*-overexpressing [rF (YehT)] to that measured in the *kdpE* overexpression [rF (KdpE)] strain. A negative (positive) value denotes a decrease (increase) in transcription level upon YehT overproduction in comparison to that seen in the KdpE overproduction strain.

[c] p significance (t-test) of single rF values.

[d] Effect of YehT-D54E or KdpE overproduction on the transcript levels of the respective genes in *E. coli* MG2 compared to control cells (*E. coli* MG2, pBAD24), as determined by Northern blot analysis. YehT-dependent, respectively KdpE-dependent, induction (YehT ↑, KdpE ↑) or repression (YehT ↓, KdpE ↓) of the gene is indicated (see Figure 2A).

TABLE S3. Internal control genes affected by the overexpression of *yehT* or *kdpE.*

gene[a]	b-number[a]	rF (YehT)[b]	rF (KdpE)[b]	log2 Ratio[b]	p[c]	function[a]
yehT	b2125	11,240	2,060	2.5	≤ 0,01	two-component system response regulator
yehU	b2126	640	1,090	-0.8	0,065	two-component system histidine kinase
kdpE	b0694	930	17,380	-4.2	0,013	two-component system response regulator
kdpD	b0695	570	7,370	-3.7	≤ 0,001	two-component system histidine kinase
kdpF	b4513	50	5,020	-6.6	≤ 0,001	subunit of P-Type ATPase potassium ion transporter
kdpA	b0698	600	10,200	-4.1	≤ 0,001	subunit of P-Type ATPase potassium ion transporter
kdpB	b0697	430	7,570	-4.2	≤ 0,001	subunit of P-Type ATPase potassium ion transporter
kdpC	b0696	630	10,130	-4.0	≤ 0,001	subunit of P-Type ATPase potassium ion transporter

[a] Gene names/b-numbers and gene product function are adopted from http://www.ecocyc.org (3) and the Affymetrix Expression Analysis Sequence Information Database (2).

[b] Log_2 ratio of transcript levels for the *yehT*- and *kdpE*-overexpression strains. Log_2 was calculated from the ratio of the mean fluorescence intensity of the respective transcript in the *yehT*-overexpressing [rF (YehT)] to that measured in the *kdpE* overexpression [rF (KdpE)] strain. A negative (positive) value denotes a decrease (increase) in transcription level upon YehT overproduction in comparison to that seen in the KdpE overproduction strain.

[c] p significance (t-test) of single rF values.

[d] Effect of YehT-D54E or KdpE overproduction on the transcript levels of the respective genes in *E. coli* MG2 compared to control cells (*E. coli* MG2, pBAD24), as determined by Northern blot analysis. YehT-dependent, respectively KdpE-dependent, induction (YehT ↑, KdpE ↑) or repression (YehT ↓, KdpE ↓) of the gene is indicated (see Figure 2A).

REFERENCES:

1. **Argaman, L., R. Hershberg, J. Vogel, G. Bejerano, E. G. H. Wagner, H. Margalit, and S. Altuvia.** 2001. Novel small RNA-encoding genes in the intergenic regions of *Escherichia coli*. Cur. Biol. **11:**941-950.
2. **Cheng, J., S. Sun, A. Tracy, E. Hubbell, J. Morris, V. Valmeekam, A. Kimbrough, M. S. Cline, G. Liu, and R. Shigeta.** 2004. NetAffx Gene Ontology Mining Tool: a visual approach for microarray data analysis. Bioinformatics **20:**1462-1463.
3. **Keseler, I. M., C. Bonavides-Martínez, J. Collado-Vides, S. Gama-Castro, R. P. Gunsalus, D. A. Johnson, M. Krummenacker, L. M. Nolan, S. Paley, I. T. Paulsen, M. Peralta-Gil, A. Santos-Zavaleta, A. G. Shearer, and P. D. Karp.** 2009. EcoCyc: A comprehensive view of *Escherichia coli* biology. Nucleic Acids Res. **37:**D464-D470.

10. Supplemental Material – Chapter 2

Supplemental Material:

Identification of a target gene and activating stimulus for the YpdA/YpdB histidine kinase/response regulator system in *Escherichia coli*

Luitpold Fried[1#], Stefan Behr[1#], and Kirsten Jung[1*]

[1]Munich Center for Integrated Protein Science (CIPSM) at the Department of Microbiology,

Ludwig-Maximilians-Universität München, 82152 Martinsried, Germany

[#] These authors contributed equally to this work

Running title: The YpdA/YpdB-system in *E. coli*

*To whom correspondence should be addressed:
Dr. Kirsten Jung
Ludwig-Maximilians-Universität München
Department Biologie I, Bereich Mikrobiologie
Großhaderner Str. 2-4
82152 Martinsried
Germany
Phone: +49-89-2180-74500
Fax: +49-89-2180-74520
E-mail: jung@lmu.de

TABLE S1. Plasmids used in this study.

Plasmid	Description	Reference or source
pRed/ET	λ-RED recombinase in pBAD24; Amp^r	Gene Bridges
pCP20	FLP-recombinase, λcI 857^+, λpR Rep^{ts}; Amp^r, Cm^r	(1)
pBAD33-Cm	Arabinose-inducible P_{BAD} promoter, pBR322 ori; Kan^r	(2)
pBAD33-*ypdB*	*6his-ypdB* cloned in the AflII and XbaI sites of pBAD33-Cm; Cm^r	This work
pBAD24	Arabinose-inducible P_{BAD} promoter, pBR322 ori; Amp^r	(2)
pBAD24-*ypdB*	*6his-ypdB* cloned in the EcoRI and XbaI sites of pBAD24; Amp^r	This work
pBAD24-*ypdB* D53E	*ypdB* D54E cloned in the NdeI and XbaI sites of pBAD24-*ypdB*; Amp^r	This work
pBAD24-*ypdB* D53N	*ypdB* D54N cloned in the NdeI and XbaI sites of pBAD24-*ypdB*; Amp^r	This work
pBAD24-*yehS*	*yehS* cloned in the NdeI and XbaI sites of pBAD24-*kdpE*; Amp^r	This work
pBAD24-*ypdA*	*ypdA* cloned in the EcoRI and XbaI sites of pBAD24; Amp^r	This work
pBAD24-*ypdA* H371Q	*ypdA* H371Q cloned in the EcoRI and XbaI sites of pBAD24; Amp^r	This work
pBAD24-*yhjX*	*yhjX-6his* cloned in the EcoRI and XbaI sites of pBAD24; Amp^r	This work
pBAD24-*ypdAB*	*ypdAB* cloned in the EcoRI and XbaI sites of pBAD24; Amp^r	This work
pBAD24-*ypdABC*	*ypdABC* cloned in the EcoRI and XbaI sites of pBAD24; Amp^r	This work
pUC19	IPTG-inducible P_{Lac} promoter, pMB1 ori, Amp^r	(3)
pUC19 $P_{yjiY\ -212/+88}$	$P_{yjiY\ -212/+88}$ cloned in the EcoRI and BamHI sites of pUC19; Amp^r	(4)
pUC19 $P_{yhjX\ -264/+36}$	$P_{yhjX\ -264/+36}$ cloned in the EcoRI and BamHI sites of pUC19; Amp^r	This work
pUC19 $P_{yhjX\ -264/-165}$	$P_{yhjX\ -264/-165}$ cloned in the EcoRI and BamHI sites of pUC19; Amp^r	This work
pUC19 $P_{yhjX\ -164/-65}$	$P_{yhjX\ -164/-65}$ cloned in the EcoRI and BamHI sites of pUC19; Amp^r	This work
pUC19 $P_{yhjX\ -64/+36}$	$P_{yhjX\ -64/+36}$ cloned in the EcoRI and BamHI sites of pUC19; Amp^r	This work
pRS415	Operon fusion vector	(5)
pRS415 $P_{yhjX\ -264/+36}$	$P_{yhjX\ -264/+36}$ cloned in the EcoRI and BamHI sites of pRS415; Amp^r	This work
pRS415 $P_{yhjX\ up\_rplmt}$	$P_{yhjX\ up\_rplmt}$ (replacement of 15 bp upstream of M1) cloned in the EcoRI and BamHI sites of pRS415; Amp^r	This work
pRS415 $P_{yhjX\ M1}$	$P_{yhjX\ M1}$ (replacement of M1) cloned in the EcoRI and BamHI sites of pRS415; Amp^r	This work
pRS415 $P_{yhjX\ spacer}$	$P_{yhjX\ spacer}$ (replacement of spacer) cloned in the EcoRI and BamHI sites of pRS415; Amp^r	This work
pRS415 $P_{yhjX\ M2}$	$P_{yhjX\ M2}$ (replacement of M2) cloned in the EcoRI and BamHI sites of pRS415; Amp^r	This work
pRS415 $P_{yhjX\ down\text{-}rplmt}$	$P_{yhjX\ down\_rplmt}$ (replacement of 15 bp downstream of M2) cloned in the EcoRI and BamHI sites of pRS415; Amp^r	This work
pRS415 $P_{yhjX\ M2S1}$	$P_{yhjX\ M2S1}$(replacement of bp 1 and 10 in M2) cloned in the EcoRI and	This work

	BamHI sites of pRS415; Amp^r	
pRS415 $P_{yhjX\ M2S2}$	$P_{yhjX\ M2S2}$(replacement of bp 1,2,9 and 10 in M2) cloned in the EcoRI and BamHI sites of pRS415; Amp^r	This work
pRS415 $P_{yhjX\ M2S3}$	$P_{yhjX\ M2S3}$ (replacement of bp 1,2,3,8,9 and 10 in M2) cloned in the EcoRI and BamHI sites of pRS415; Amp^r	This work
pBBR1-MCS5-TT-RBS-*lux*	*luxCDABE* and terminators lambda *T0 rrnB1 T1* cloned into pBBR1-MCS5 for plasmid-based transcriptional fusions; Gm^r	(6)
pBBR *yhjX-lux*	$P_{yhjX\ -264/+36}$ cloned in the BamHI and EcoRI sites of pBBR1-MCS5-TT-RBS-*lux*; Gm^r	This work

TABLE S2. Oligonucleotides used in this study

Oligonucleotide	#	Oligonucleotide Sequence (5´-3´)	Description
Plasmid or strain construction			
YpdB NdeI sense		AACATATGGTGAAAGTCATCATTGTTGAA	pBAD24-*ypdB*
YpdB XbaI antisense		CCTCTAGATTAAAGATGCATTAACTGGCG	pBAD24-*ypdB*, pBAD24-*ypdAB*
ypdB B53E sense		GCCATTTTTCTGGAAATCAATATTCCG	pBAD24-*ypdB*-D53E
ypdB B53E antisense		CGGAATATTGATTTCCAGAAA AATGGC	pBAD24-*ypdB*-D53E
ypdB D53N sense		ATAACCGCGTCGACGCCATTT TTCTGAATATCA ATATTCCGTCGCTGG ATGGCG T	pBAD24-*ypdB*-D53N
ypdB D53N anti		ACGCCATCCAGCGACGGAATATTGATATTCAGAAAAATGGCGTCGAC GCGGTTAT	pBAD24-*ypdB*-D53N
yehS NdeI sense		ATGCGCCATATGCTAAGTAACGATATTCTGC	pBAD24-*yehS*
yehS XbaI antisense		CTCTCTAGATTAGCCTTTTTTCACATGCT	pBAD24-*yehS*
yhjX EcoRI sense		CAGGAGGAATTCATGACACCTTCAAATTATCAGC	pBAD24-*yhjX*
yhjX NdeI anti		GGAATTCCATATGAAGGGAGCCATGCGCCTCACGCAAC	pBAD24-*yhjX*
YpdA EcoRI sense		CCGAATTCGTGCACGAAATATTCAACATG	pBAD24-*ypdA*, pBAD24-*ypdAB*, pBAD24-*ypdABC*, pBAD24-*ypdA* H371Q
YpdA NdeI antisense		AACATATGAAGCAATAACGTAGCCTGTGA	pBAD24-*ypdA*, pBAD24-*ypdA* H371Q
YpdC XbaI antisense		CCTCTAGATTAGCCCTGAAAACGGGCGCT	pBAD24-*ypdABC*
ypdA H371Q sense		TCGCGCCCTGCAAAGCAAAATTAATCCCCAGTTTCTGTTTAACGCTCT GAACGCTATTTCA	pBAD24-*ypdA* H371Q
ypdA H371Q anti		TGAAATAGCGTTCAGAGCGTTAAACAGAAACTGGGGATTAATTTTGCT TTGCAGGGCGCGA	pBAD24-*ypdA* H371Q
pBAD24 anti		CAAATTCTGTTTTATCAGACCGCTTCTGCG	pBAD24 sequencing
pBAD24 sense		TCGCAACTCTCTACTGTTTCTCCATA	pBAD24 sequencing
rev24		TTCACACAGGAAACAGCTATGACC	pUC19 sequencing, labeling EMSA
uni24		ACGACGTTGTAAAACGACG	pUC19 sequencing
up yhjX 300bp BamHI sense		AATCCGGATCCCTAACTCAGGCAGAAAATACCA	pBBR *yhjX-lux*
up yhjX EcoRI anti		ATACCGGAATTCGGCAGTATTCCTGCAGTAATAAAAAG	pBBR *yhjX-lux*
Up YpdA		AGCCTTCAGGTTACCTATCATAGAGGTTTAATCCTTATTCAGAGTCAC CCAATTAACCCTCACTAAAGGGCGG	*E. coli* MG20 construction
Low YpdC		GATGCACAAAGTATCCTGACGCTGCTGGAAACAGAATTAACCTTCTGA CGTAATACGACTCACTATAGGGCTCG	*E. coli* MG20 construction
YpdBC-rpsL-neo-up		AACAGGAACTGAGCTGGCTAATTAAAGAGCACAGCCAGATGGAGATT GTCGGCACCTTTGGGCCTGGTGATGATGGCGGGATCG	*E. coli* MG21 construction
YpdBC-rpsL-neo-down		GCAAGATGCACAAAGTATCCTGACGCTGCTGGAAACAGAATTAACCT TCTGACGTCAGAAGAACTCGTCAAGAAGGCG	*E. coli* MG21 construction
RED-Kan anti		CGAGACTAGTGAGACGTGCTAC	control primer
RED-Kan sense		TATCAGGACATAGCGTTGGCTACC	control primer
ypdB sense		CGTTACTTAGCATGAGGCCTT	control primer
ypdB +84 sense		TGTGAGCCTGATAGTTACACC	control primer
ypdA +350 s		CCGGACCGTCCGAGCGACGCT	control primer
ypdA +50 s		AGCCTTCAGGTTACCTATCAT	control primer
ypdC + 50 a		GATGCACAAAGTATCCTGACG	control primer
ypdC + 350 a		CGCACTGAACATCCGTTTGAG	control primer

down-ypdB-rpsl - D53	CACGCGGTGATGAACACAATAAACGGTTTATGGGCGAACTGGCTGAT GTTTCAGAAGAACTCGTCAAGAAGGCG	*E. coli* MG 24 / MG 25 construction
up ypdA rpsl neo	AATGCTTATCTGCCTGTTCTTTCTCATCCGTATCCGCCTGTTTCGCGA ACGGCCTGGTGATGATGGCGGGATCG	*E. coli* MG23 construction
down ypdA rspl neo	AATGTAAAACGCAATTTCCGTCCCCGGCTCCAGGCGGCGGATATGCA GCCTCAGAAGAACTCGTCAAGAAGGCG	control primer
ypdA sense	GTGCACGAAATATTCAACATG	control primer
ypdA anti	TCAAAGCAATAACGTAGCCT	control primer
YpdA+up50bp sense	AGCCTTCAGGTTACCTATCATAGAGGTTTAATCCTTATTCAGAGTCAC CCGTGCACGAAATATTCAACATG	*E. coli* MG 23 construction
YpdA-down50bp anti	TGCCAGGAATTCGTCTTCAACAATGATGACTTTCACAATATCACTCCG GCTCAAAGCAATAACGTAGCCTGT	*E. coli* MG 23 construction
YpdB+up50bp sense	ACCCCAGTCGCCTCACAGGCTACGTTATTGCTTTGAGCCGGAGTGAT ATTGTGAAAGTCATCATTGTTGAAGA	*E. coli* MG 24 / MG 25 construction
YpdB-down50bp anti	AAAAATTGTTGATCGGCGGGCAAGCCTGGTGCTTTCATGAAAGTTCC CGATTAAAGATGCATTAACTGGCGAAAT	*E. coli* MG 24 / MG 25 construction
up yhjX	TATGGTTGTCGGCAGAGATTTTTCCTTTTTATTACTGCAGGAATACTG CCAATTAACCCTCACTAAAGGGCG	*E. coli* MG26 construction
down yhjX	ATGCGTTTGATGCACACGGAAGCTGAAGCCCAGTAGCTCGCGGCTG AGCATAATACGACTCACTATAGGGCTC	*E. coli* MG26 construction
yhjX-200	GCAAAGGGAAAAAGTGTGGGGA	control primer

Northern Blot DNA probes

cpxP anti	CTACTGGGAACGTGAGTTGCT	*cpxP* probe
cpxP sense	ATGCGCATAGTTACCGCTGCC	*cpxP* probe
entC anti	TTAATGCAATCCAAAAACGTT	*entC* probe
entC sense	ATGGATACGTCACTGGCTGAG	*entC* probe
entE anti	TGCCAAACACCTGCTGCAACT	*entE* probe
entE sense	ATGAGCATTCCATTCACCCGC	*entE* probe
fecA anti	GCAGGCTGTTGAAGGTGTGCA	*fecA* probe
fecA sense	ATGACGCCGTTACGCGTTTTT	*fecA* probe
fecB anti	TCATTTCACAACGGTAAGCGG	*fecB* probe
fecB sense	ATGTTGGCATTTATCCGTTTT	*fecB* probe
fhuA anti	GCAGGTTCTGACGCACAGTAA	*fhuA*probe
fhuA sense	ATGGCGCGTTCCAAAACTGCT	*fhuA*probe
fhuF anti	TCATTTCAGCGTACAATCGCC	*fhuF* probe
fhuF sense	ATGGCCTATCGTTCCGCACCG	*fhuF* probe
guaC anti	TTACAGGTTGTTGAAGATGCG	*guaC* probe
guaC sense	ATGCGTATTGAAGAAGATCTG	*guaC* probe
iraP anti	TTACTGACGAGGATGCTTCAA	*iraP* probe
iraP sense	ATGAAAAATCTCATTGCTGAG	*iraP* probe
rpoD anti	AATCGTCCAGGAAGCTACGCAGC	*rpoD* probe
rpoD sense	ATGGAGCAAAACCCGCAGTCAC	*rpoD* probe
yahM anti	CTACGTAATCAACCTGATTTG	*yahM* probe
yahM sense	ATGGCGGTCCAACTTTTCAAA	*yahM* probe
yehS anti	TTAGCCTTTTTTCACATGCTG	*yehS* probe
yehS sense	ATGCTAAGTAACGATATTCTG	*yehS* probe
ygbK anti	TTACCCACGGCACGCCGGGGAAAT	*ygbK* probe
ygbK sense	ATGATCAAGATTGGCGTTATC	*ygbK* probe
ygbL anti	TTAACTCCTTAATTCCGCAAT	*ygbL* probe
ygbL sense	ATGAGCGATTTCGCAAAAGTA	*ygbL* probe
yhjX anti	CAAAGAACTCACTGACCAGTG	*yhjX* probe
yhjX sense	ATGACACCTTCAAATTATCAG	*yhjX* probe
yjiY anti	TGATGAACAGGAACGGGAACA	*yjiY* probe
yjiY sense	ATGGATACTAAAAAGATATTC	*yjiY* probe
ynjH anti	TTATGGCTTTACGCGCCGCCA	*ynjH* probe
ynjH sense	GTGAGTCGAGCATTGTTCGCC	*ynjH* probe
ypdB anti	TTAAAGATGCATTAACTGGCG	*ypdB* probe

ypdB sense	GTGAAAGTCATCATTGTTGAA	*ypdB* probe
EMSA/footprint		
6-FAM uni24	[6-FAM]ACGACGTTGTAAAACGACGGCCAG	EMSA labeling DNA-fragments
yhjX 1 sense	TTGAATTCTTCTGATGGCATTTCATG	pUC19 $P_{yhjX\ -64/+36}$
yhjX 1 anti	TTGGATCCGGCAGTATTCCTGCAGTA	pUC19 $P_{yhjX\ -264/+36}$, pRS415 $P_{yhjX\ -264/+36}$ + derivates
yhjX 2 sense	TTGAATTCTAACAATAGTTGTGGCGA	pUC19 $P_{yhjX\ -164/-65}$
yhjX 2 anti	TTGGATCCCGGAATGAAATGCCTTAG	pUC19 $P_{yhjX\ -164/-65}$
yhjX 3 sense	TTGAATTCCTAACTCAGGCAGAAAAT	pUC19 $P_{yhjX\ -264/-165}$, pUC19 $P_{yhjX\ -264/+36}$
yhjX 3 anti	TTGGATCC TTTAATGGTTTCAATTGT	pUC19 $P_{yhjX\ -264/-165}$
yjiY-5P-1 anti	TTTTTTGGATCCAGTAAAACCTGGCATGTA	pUC19 $P_{yjiY\ -212/+88}$
yjiY-5P-3 sense	TTTTTTGAATTCCGCCGAGTGAATTTTATTCA	pUC19 $P_{yjiY\ -212/+88}$
In vivo reporter		
upstream-replacement as	GGCTGGACTTCCGTCATGACGCGACAATTATTC	pRS415 $P_{yhjX\ up\_rplmt}$
upstream-replacement s	GACGGAAGTCCAGCCGGCATTTCATTCCGTTCT	pRS415 $P_{yhjX\ up\_rplmt}$
motif 1 replacement as	CGTCCCGTAATTAGTTCAGGAATGAATG	pRS415 $P_{yhjX\ M1}$
motif 1 replacement s	TTACGGGACGTCCGTTCTGATGGCATTT	pRS415 $P_{yhjX\ M1}$
spacer replacement as	CGACTCCATTCATGAAATGCCTTAGTTCA	pRS415 $P_{yhjX\ spacer}$
spacer replacement s	GAATGGAGTCGGGCATTTCATGCCGTTTT	pRS415 $P_{yhjX\ spacer}$
motif 2 replacement as	CGTCCCGTAAATCAGAACGGAATGAAAT	pRS415 $P_{yhjX\ M2}$
motif 2 replacement s	TTACGGGACGGCCGTTTTTCCCCAGGCA	pRS415 $P_{yhjX\ M2}$
downstream-replacement as	AGTTTTCCCCCATTAATGAAATGCCATCAGAAC	pRS415 $P_{yhjX\ down\_rplmt}$
downstream-replacement s	TAATGGGGGAAAACTGCATAAAGTGCACTTCGT	pRS415 $P_{yhjX\ down\_rplmt}$
motif shortening 1 as	ATCAGAACGGACTGAAATGCATTAGTTCAGGAATGAATG	pRS415 $P_{yhjX\ M2\ G/T}$
motif shortening 2 as	ATCAGAACGGACGGAAATGAATTAGTTCAGGAATGAATG	pRS415 $P_{yhjX\ M2\ GG/AT}$
motif shortening 3 as	ATCAGAACGGACGTAAATTAATTAGTTCAGGAATGAATG	pRS415 $P_{yhjX\ M2\ GGC/CAT}$
motif shortening 1 s	TCCGTTCTGATTGCATTTCAGGCCGTTTTTCCCCAGGCA	pRS415 $P_{yhjX\ M2\ G/T}$
motif shortening 2 s	TCCGTTCTGATTTCATTTCCGGCCGTTTTTCCCCAGGCA	pRS415 $P_{yhjX\ M2\ GG/AT}$
motif shortening 3 s	TCCGTTCTGATTTAATTTACGGCCGTTTTTCCCCAGGCA	pRS415 $P_{yhjX\ M2\ GGC/CAT}$

TABLE S3: Influence of C-sources and additives on *yhjX* expression. Strain and cultivation conditions were the same as described in Figure 4B.

Medium	Additional C-source	Concentration C-source	Additive	Concentration additive	Average of max. *yhjX* expression [RLU/OD_{600}]	Standard deviation of max. *yhjX* expression [RLU/OD_{600}]
LB medium	Glucose	0.4%	Pyruvate	20 mM	41,710	3,020
LB medium	Pyruvate	20 mM			38,810	1,270
LB medium	-	-	-	-	35,450	3,670
LB medium	Glucose	0.4%	-	-	10,820	1,910
M9 medium	Pyruvate	20 mM	-	-	479,070	46,060
M9 medium	Yeast extract	0.5%	-	-	38,240	4,460
M9 medium	Gluconic acid	0.4%	-	-	7,260	620
M9 medium	Glucuronic acid	0.4%	-	-	4,120	860
M9 medium	Lactate	20 mM	-	-	1,000	160
M9 medium	Phosphoenol pyruvate	20 mM	-	-	370	80
M9 medium	Glycerol	0.4%	-	-	360	70
M9 medium	L-Serine	20 mM	-	-	360	50
M9 medium	Acetate	0.4%	-	-	320	30
M9 medium	Lactose	0.4%	-	-	300	20
M9 medium	Casamino acids	0.4%	-	-	260	30
M9 medium	L-Proline	20 mM	-	-	260	40
M9 medium	Fumarate	20 mM	-	-	250	50
M9 medium	Mannose	0.4%	-	-	250	50
M9 medium	Succinate	0.4%	-	-	250	40
M9 medium	Galactose	0.4%	-	-	230	30
M9 medium	Oxaloacetate	20 mM	-	-	230	30
M9 medium	Peptone	0.4%	-	-	220	60
M9 medium	L-Asparte	20 mM	-	-	210	40
M9 medium	Mannitol	0.4%	-	-	210	40
M9 medium	Fructose	0.4%	-	-	200	40
M9 medium	Xylose	0.4%	-	-	200	50
M9 medium	Maltose	0.4%	-	-	170	40
M9 medium	Glucose	0.4%	-	-	160	150
M9 medium	L-Glutamate	0.4%	-	-	130	40
M9 medium	Tryptone	0.4%	-	-	10	10
M9 medium	Cas amino acids	0.4%	Glucose	0.4%	22,640	520
M9 medium	Cas amino acids	1.5%	PIPES (pH 5.5) + Glycerol	20 mM + 0.8%	9,910	720
M9 medium	Cas amino acids	1.5%	PIPES (pH 7.0) + Glycerol	20 mM + 0.8%	7,620	240
M9 medium	Pyruvate	20 mM	Fumarate	20 mM	528,780	66,840
M9 medium	Pyruvate	20 mM	Glucose	20 mM	281,270	28,830
M9 medium	Pyruvate	20 mM	Glucose	1 mM	270,880	41,130

M9 medium	Pyruvate	20 mM	Lactate + PIPES (pH 7.0)	20 mM + 20 mM	240,000	16,200
M9 medium	Pyruvate	20 mM	Glucose	5 mM	201,370	6,160
M9 medium	Pyruvate	20 mM	Acetate	20 mM	165,180	20,310
M9 medium	Succinate	0.4%	Pyruvate	20 mM	274,060	17,380
M9 medium	Succinate	0.4%	Fumarate	20 mM	9,940	1,130
M9 medium	Succinate	0.4%	Lactose	0.4%	3,350	790
M9 medium	Succinate	0.4%	Guanidine hydrochloride	1 mg/ml	660	390
M9 medium	Succinate	0.4%	Methanol	1%	600	480
M9 medium	Succinate	0.4%	Fosfomycin	1 µg/ml	570	480
M9 medium	Succinate	0.4%	Crystal violet	0.05 µg/µl	560	450
M9 medium	Succinate	0.4%	D-Leucine	20 mM	540	40
M9 medium	Succinate	0.4%	L-Histidine	20 mM	530	380
M9 medium	Succinate	0.4%	Sulfamethazine	0.5 µg/ml	510	30
M9 medium	Succinate	0.4%	Arsenate	20 µg/ml	500	190
M9 medium	Succinate	0.4%	Imipenem	0.05 µg/ml	480	210
M9 medium	Succinate	0.4%	Plumbagin	8 µg/ml	470	240
M9 medium	Succinate	0.4%	D-Argine	20 mM	410	430
M9 medium	Succinate	0.4%	D-Proline	20 mM	410	180
M9 medium	Succinate	0.4%	Maltose	0.4%	390	60
M9 medium	Succinate	0.4%	Xylose	0.4%	380	50
M9 medium	Succinate	0.4%	Antimony(III)chloride	5 µg/ml	370	140
M9 medium	Succinate	0.4%	Hdroxyurea	100 µg/ml	350	200
M9 medium	Succinate	0.4%	Paromomycin	0.01 µg/ml	350	150
M9 medium	Succinate	0.4%	Paromomycin	0.005 µg/ml	340	130
M9 medium	Succinate	0.4%	5,7-Dichloro-8-hydroxyquinaldine	1 µg/ml	330	360
M9 medium	Succinate	0.4%	D-Tyrosine	20 mM	330	110
M9 medium	Succinate	0.4%	Oxalate	30 mM	330	220
M9 medium	Succinate	0.4%	Propanol	1%	330	330
M9 medium	Succinate	0.4%	Thiamphenicol	1 µg/ml	300	160
M9 medium	Succinate	0.4%	Polymyxin B	0.005µg/ml	290	130
M9 medium	Succinate	0.4%	Deoxycholate	100 µg/ml	280	340
M9 medium	Succinate	0.4%	Lactulose	30 mM	280	250
M9 medium	Succinate	0.4%	Natriumphosphat (pH 7)	200 mM	280	250
M9 medium	Succinate	0.4%	7-Hydoxycoumarin	10µg/ml	270	220
M9 medium	Succinate	0.4%	Tobramycin	0.01 µg/ml	270	460
M9 medium	Succinate	0.4%	Arsenite	10 µg/ml	260	10
M9 medium	Succinate	0.4%	L-Alanine	20 mM	260	60
M9 medium	Succinate	0.4%	Iodacetic acid	10 µg/ml	250	240
M9 medium	Succinate	0.4%	L-Leucine	20 mM	240	230
M9 medium	Succinate	0.4%	Lactate	20 mM	230	90
M9 medium	Succinate	0.4%	Deoxycholate	50 µg/ml	210	220
M9 medium	Succinate	0.4%	L-Arginine	20 mM	210	180
M9 medium	Succinate	0.4%	L-Isoleucine	20 mM	200	40
M9 medium	Succinate	0.4%	L-Tyrosine	20 mM	200	190

M9 medium	Succinate	0.4%	N-Acetyl-Glucosamine	0.4%	170	40
M9 medium	Succinate	0.4%	Deoxycholate	500 µg/ml	160	280
M9 medium	Succinate	0.4%	NaCl	2%	160	40
M9 medium	Succinate	0.4%	PIPES (pH 7.0) + Oxalate	20 mM + 30 mM	160	40
M9 medium	Succinate	0.4%	Apramycin	0.005 µg/ml	150	140
M9 medium	Succinate	0.4%	Benzoate	30 mM	140	120
M9 medium	Succinate	0.4%	Ethanol	5%	130	230
M9 medium	Succinate	0.4%	Peptidoglycan E. coli	1/20 fold dilution	130	100
M9 medium	Succinate	0.4%	PIPES (pH 7.0) + Mitomycin	20 mM + 0.3 µg/ml	130	30
M9 medium	Succinate	0.4%	D-Cycloserine	0,5 µg/ml	120	190
M9 medium	Succinate	0.4%	D-Serine	20 mM	120	110
M9 medium	Succinate	0.4%	Ethanol	1%	120	200
M9 medium	Succinate	0.4%	Gly-Gly	20 mM	100	300
M9 medium	Succinate	0.4%	L-Asparagine	20 mM	100	140
M9 medium	Succinate	0.4%	L-Threonine	20 mM	100	180
M9 medium	Succinate	0.4%	PIPES (pH 7.0)	20 mM	90	20
M9 medium	Succinate	0.4%	D-Alanine	20 mM	80	130
M9 medium	Succinate	0.4%	L-Glutamate	20 mM	80	130
M9 medium	Succinate	0.4%	L-Proline	20 mM	80	140
M9 medium	Succinate	0.4%	Chlorambucil	50 µg/ml	70	120
M9 medium	Succinate	0.4%	Ethanol	2%	70	110
M9 medium	Succinate	0.4%	L-Glycine	20 mM	70	120
M9 medium	Succinate	0.4%	Phenyl-methylsufonyl-fluorid	200 µg/ml	60	110
M9 medium	Succinate	0.4%	PIPES (pH 7.0) + Formate	20 mM + 30 mM	60	20
M9 medium	Succinate	0.4%	L-Serine	20 mM	50	80
M9 medium	Succinate	0.4%	Peptidoglycan Bacillus	1/20 fold dilution	50	30
M9 medium	Succinate	0.4%	Peptidoglycan Lactobacillus	1/20 fold dilution	50	90
M9 medium	Succinate	0.4%	Amitriptylin	10 µg/ml	40	40
M9 medium	Succinate	0.4%	Formate	30 mM	40	70
M9 medium	Succinate	0.4%	L-Aspartate	20 mM	40	30
M9 medium	Succinate	0.4%	Methylglyoxal	0,7 mM	40	10
M9 medium	Succinate	0.4%	Peptidoglycan Lactobacillus	1/2000 fold dilution	40	20
M9 medium	Succinate	0.4%	D-Ala-D-Ala	20 mM	30	20
M9 medium	Succinate	0.4%	Dulcitol	0.4%	30	50
M9 medium	Succinate	0.4%	Methylglyoxal	0,2 mM	30	10
M9 medium	Succinate	0.4%	Peptidoglycan Bacillus	1/2000 fold dilution	30	40
M9 medium	Succinate	0.4%	PIPES (pH 7.0) + Benzoate	20 mM + 30 mM	20	10

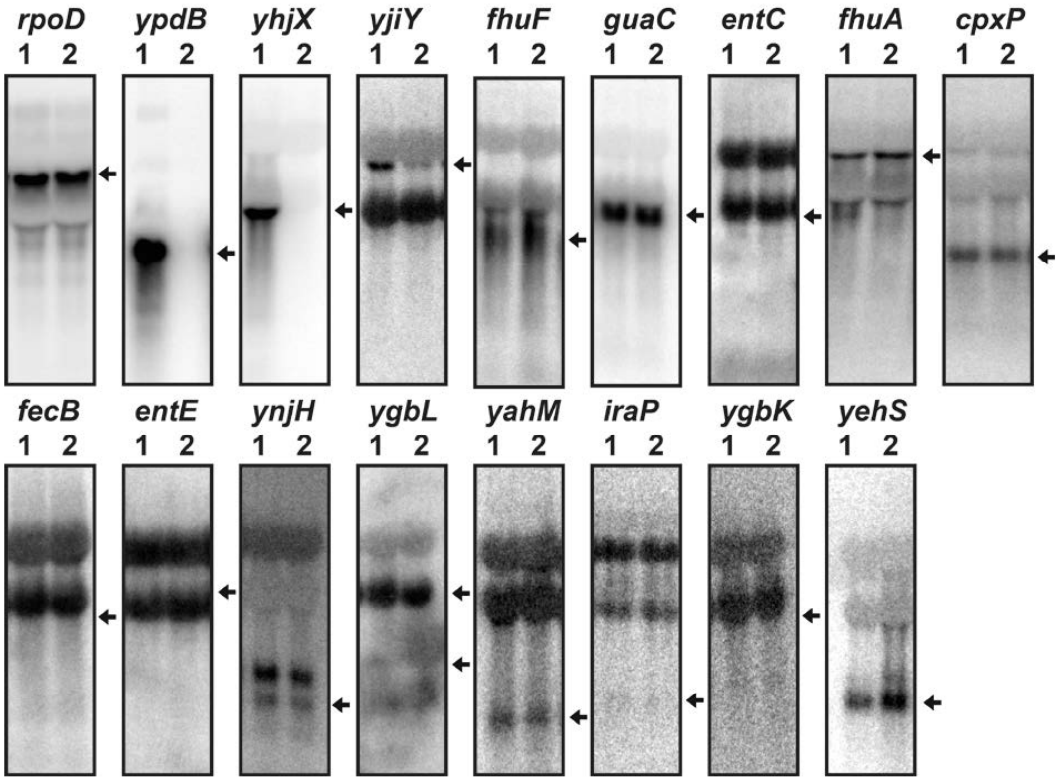

FIG. S1. Evaluation of potential YpdB target genes. A) Northern blot analysis was used to measure the effect of overproduction of YpdB on the expression of the genes identified by transcriptome analysis (see Table 1) and *rpoD* (control) in *E. coli* MG21 (Δ*ypdB*). The expression levels of these genes were also assessed in the *E. coli* strain MG21 (Δ*ypdB*) in the absence of YpdB (*E. coli* MG21 transformed with the empty pBAD24 vector) (lanes 2) or upon overproduction of YpdB (lanes 1). 20 µg of total RNA was loaded per lane, and the transcripts were detected with the corresponding gene-specific DNA probes. Transcripts of the corresponding genes are marked by an arrow.

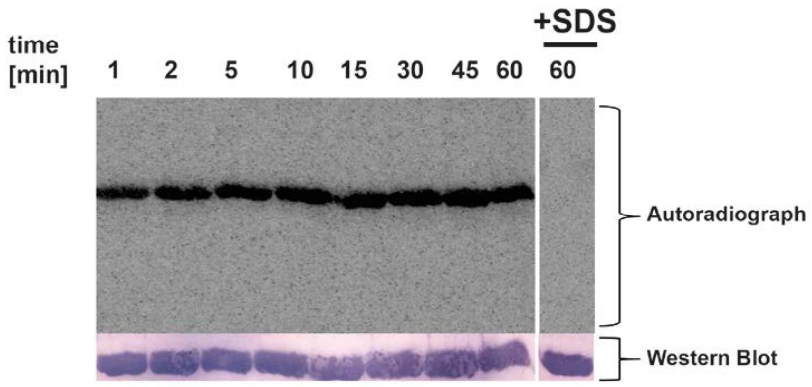

Fig. S2. In vitro phosphorylation of YpdB. Purified YpdB-6His was mixed with phosphorylation buffer. Phosphorylation was started by adding a mixture of [γ–^{32}P]acetyl phosphate and $MgCl_2$. At the indicated times, the reaction was stopped by adding SDS-sample buffer, the samples were subjected to SDS-PAGE and Semi Dry Western Blotting. As negative control, protein was denatured by adding SDS-sample before the reaction was started (+SDS). Phosphorylated YpdB was detected by autoradiography using a phosphor screen and a PhosphorImager Storm. The autoradiograph is representative of three independent experiments.

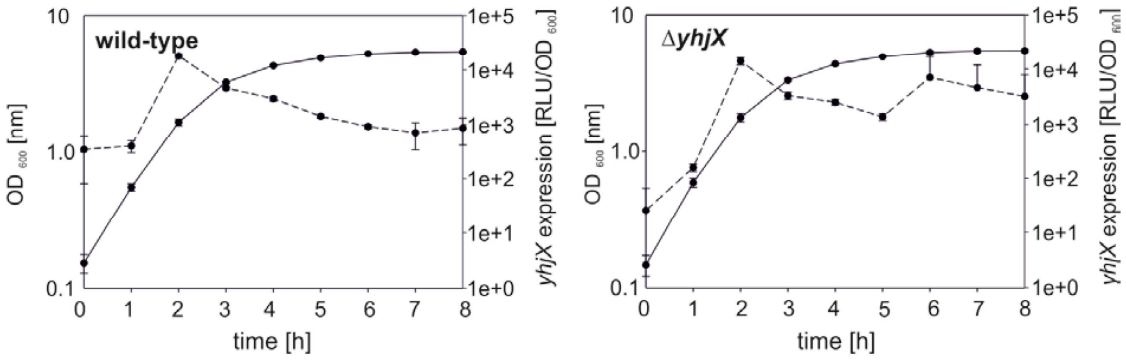

Fig. S3. *yhjX* induction is independent of YhjX feedback regulation. *Escherichia coli* MG1665 (wild-type) and MG26 (Δ*yhjX*) were transformed with pBBR *yhjX*-lux and grown aerobically in LB medium. Growth and luciferase activity were monitored continuously. The maximal luciferase activity normalized to an optical density of 1 (RLU/OD600) was used as a measure of the degree of induction of *yhjX*. Data were obtained from at least three independent experiments, and average values were used for calculations.

References:

1. **Cherepanov PP, Wackernagel W.** 1995. Gene disruption in *Escherichia coli*: TcR and KmR cassettes with the option of Flp-catalyzed excision of the antibiotic-resistance determinant. Gene **158:**9-14.
2. **Guzman L, Belin D, Carson M, Beckwith J.** 1995. Tight regulation, modulation, and high-level expression by vectors containing the arabinose PBAD promoter. J. Bacteriol. **177:**4121-4130.
3. **Yanisch-Perron C, Vieira J, Messing J.** 1985. Improved M13 phage cloning vectors and host strains: nucleotide sequences of the M13mp18 and pUC19 vectors. Gene **33:**103 - 119.
4. **Kraxenberger T, Fried L, Behr S, Jung K.** 2012. First insights into the unexplored two-component system YehU/YehT in *Escherichia coli*. J. Bacteriol. **194:**4272-4284.
5. **Simons R, Houman F, Kleckner N.** 1987. Improved single and multicopy lac-based cloning vectors for protein and operon fusions. Gene **53:**85-96.
6. **Godeke J, Heun M, Bubendorfer S, Paul K, Thormann KM.** 2011. Roles of Two *Shewanella oneidensis* MR-1 Extracellular Endonucleases. Appl Environ Microbiol **77:**5342-5351.

Acknowledgements

Abschließend möchte ich die Gelegenheit nutzen und mich bei all den Menschen, die zum Gelingen dieser Arbeit beigetragen haben, herzlich bedanken.

Mein ganz besonderer Dank gilt dabei zunächst Fr. Prof. Dr. Kirsten Jung für die Aufnahme in ihre Arbeitsgruppe und die Möglichkeit das YehUT-Projekt aktiv mit zu gestalten. Es war ein faszinierendes Erlebnis dieses Projekt quasi von Anfang begleiten zu können und hat vor allem unglaublich viel Spaß gemacht! Auf diesem Weg: Vielen Dank für das entgegengebrachte Vertrauen, die vielen anregenden Diskussionen und die erstklassige Betreuung!

Ebenfalls nicht weniger herzlich möchte ich mich bei Prof. Dr. Thorsten Mascher bedanken, der sich trotz vollen Terminkalenders als Zweitgutachter für meine Arbeit zur Verfügung gestellt hat.

Darüber hinaus vielen Dank an Hr. Prof. Dr. Jung, PD Ralf Heermann und Dr. Frank Landgraf für die große Diskussionsbereitsschaft und die stets sehr gute Zusammenarbeit.

Zweifelsohne, mein größter Dank gilt allen ehemaligen und gegenwärtigen Mitgliedern des YehUT-Projekts: Tobias K., Luitpold F., Tobias B. und Ivica K. sowie den nicht weniger entbehrlichen Teilzeitkräften: Sonja K., Michael R., Nicola L., Larissa R., Kerstin D., Wolfram V. und Corinna S.

Ebenfalls ein ganz, ganz großes Dankeschön geht an alle anderen Mitglieder der Arbeitsgruppen Jung und Heermann! Vielen Dank für die vielfältige Unterstützung, sowohl auf wissenschaftlicher, als auch menschlicher Ebene: an Sophie Bu., Susi U. & Martina R. für unsere g'mütliche Büroatmosphäre, an Sophie Br. für's *Coffee?*-Fragen, an Jürgen L. für die abenteuerlich-wissenschaftlichen Diskussionen im *Bear Canyon* und natürlich auch an Hannah S., Angela G., Yang W., Laure P., Matthias R., Magdalena M., Susi Br. und Felix B. sowie alle *Ehemaligen* für euren Beistand in- und vor allem außerhalb des Labors.

Zu guter Letzt:

Vielen Dank an all euch *Akteure im Hintergrund*! Euer Anteil an dieser Arbeit ist größer als ihr wahrscheinlich glaubt. Und mein Wortschatz viel zu limitiert, als dass ich das auf einer, zehn oder hundert Seiten jemals beschreiben könnte! Deshalb schlicht: Danke!

Curriculum Vitae

Personal data:

Name:	Stefan Maximilian Behr
Date of birth:	19.08.1983
Place of birth:	Gunzenhausen
Profession:	Biologist (Diplom-Biologe)

Eduction and University:

since 11/2009	PhD thesis in the research group of Prof. Dr. Kirsten Jung at the Department of Biology, Microbiology, Ludwig-Maximilians-Universität München, Topic: "The LytS/LytTR-like histidine kinase/response regulator systems in *Escherichia coli* and their function in carbon source selectivity."
10/2009	Graduation (Diploma) Diploma thesis in the research group of Prof. Dr. Kirsten Jung at the Department of Biology, Microbiology, Ludwig-Maximilians-Universität München, Topic: "Expressionsanalyse YehU/YehT-regulierter Gene in *Escherichia coli*."
WS/2004 - SS/2009	Study of Biology at the Ludwig-Maximilians-Universität München and the Universität Bayreuth
06/2003	University-entrance diploma (Abitur)

Publications:

S. Behr, L. Fried and K. Jung, (2014), Identification of a novel nutrient sensing histidine kinase/response regulator network in *Escherichia coli*. *J. Bacteriol*. 196(11):2023-2029

L. Fried, S. Behr and K. Jung, (2013), Identification of a target gene and activating stimulus for theYpdA/YpdB histidine kinase/response regulator system in *Escherichia coli*. *J. Bacteriol*. 195(4):807-815

T. Kraxenberger, L. Fried, S. Behr and K, Jung, (2012), First Insights into the Unexplored Two-Component System in *Escherichia coli*. *J. Bacteriol*. 194(16):4272-4284

K. Jung, L. Fried, S. Behr and R. Heermann, (2012), Histidine kinases and response regulators in networks. *Curr. Opin. Microbiol.* 15(2):118-124

Conferences:

2014	*3rd Mol Micro Meeting Würzburg*, Würzburg, Germany (May, 7th-9th, 2014)
2013	*BLAST XII Meeting* on Bacterial Locomotion and Signal Transduction, Tucson, Arizona, USA (January, 20th – 25th, 2013)
2012	*Annual conference of the Association for General and Applied Microbiology* (VAAM), Tübingen, Germany (March, 18th-21st, 2012)
2011	*Annual conference of the Association for General and Applied Microbiology* (VAAM), Karlsruhe, Germany (April, 3rd-6th, 2011)
2010	28. Symposium *"Mechanisms of Gene Regulation" of the VAAM study group "regulation and signal transduction in prokaryotes"*, Neustadt Germany (October, 7th – 9th, 2010)
2010	*Annual conference of the Association for General and Applied Microbiology* (VAAM), Hannover, Germany (March, 28th-31st, 2010)

Collaborations:

Mass spectrometric analysis	Prof. Axel Imhof, Marc Borath, and Tilman Schlunck from the Biomedical Center at the Ludwig-Maximilians-Universität, München, Protein analysis unit at the Adolf-Butenandt-Institute (ZfP), Munich, Germany
Bioinformatical analysis of transcriptome data	Dr. Achim Tresch and Dr. Björn Schwab from the Gene Center at the Ludwig-Maximilians-Universität, München, Munich, Germany
Crystallography and protein structure	Prof. Dr. Karl-Peter Hopfner, and Dr. Gregor Witte from the Gene Center at the Ludwig-Maximilians-Universität, München, Munich, Germany