Dissertation

Cross-talk between two-component systems in *Escherichia coli*

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May 2016

Dissertation
submitted to the
Combined Faculties for the Natural Sciences and for Mathematics
of the Ruperto-Carola University of Heidelberg, Germany
for the degree of
Doctor of Natural Sciences

presented by

Diplom-Biologin Miriam Fischer
born in Frankfurt am Main
Oral examination: August 2016

Cross-talk between two-component systems in *Escherichia coli*

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Acknowledgements

I would like to thank Prof. Dr. Victor Sourjik for his supervision and support during the last years. I also want to thank my thesis advisory committee members PD Dr. Axel Mogk and Prof. Dr Rüdiger Hell for valuable advice and input.

I would to thank the present and former members of the Sourjik lab, who are always very helpful and willing to share their experience and an occasional banter with me.

I am grateful for the encouragement by my friends and family. I especially want to thank my parents for their consistent support and love.

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Zusammenfassung

Mikroorganismen sind einer Vielzahl von Umweltbedingungen ausgesetzt. Sie nutzen Zweikomponenten-Signalsysteme um diese zu erfassen und ihren Stoffwechsel anzupassen. Zweikomponentensysteme kommen in Genomen von Bakterien, Archaeen und niederen Eukaryoten sehr häufig vor. Sie bestehen aus einer Sensor-Histidinkinase und einem 'Response Regulator', der als Transkriptionsfaktor die Genexpression reguliert. Eine Anregung der Signalübertragung durch Zweikomponentensysteme führt zur Autophosphorylierung der Histidinkinase mit nachfolgender Phosphoryl-gruppenübertragung auf den 'Response Regulator'. Ähnlichkeiten sowohl in der Struktur als auch der Proteinsequenzen verschiedener Komponenten können eine Signalverknüpfung zwischen verschiedenen Zweikomponentensystemen in unterschiedlichen Abschnitten des Signalübertragungswegs herbeiführen. Während dieser 'cross-talk' zwischen nicht-verwandten Komponenten der Zweikomponentensysteme *in vitro* untersucht wurde, ist über das *in vivo* Verhalten wenig bekannt. In dieser Arbeit haben wir *in vivo* Wechselwirkungen der Zweikomponentensysteme in dem Enterobakterium *Escherichia coli* mit Hilfe von FRET Mikroskopie identifiziert, und die physiologisch bedeutsamen Wechselwirkungen der nicht-verwandten Komponenten des CusS/ CusR and YedV/ YedW Zweikomponentensystems beschrieben. Unsere Studien mit 'promoter-reportern', sowie Transkriptionsanalysen haben zwei Ebenen der Signalkopplung nachgewiesen, eine zwischen den Histidinkinasen und den nicht-verwandten 'response regulatoren', eine weitere auf dem Genexpressions-induktionsniveau. Unsere Ergebnisse vermitteln somit ein genaues Verständnis der gegenseitigen Regulation der Zielgene und des 'cross-talks' zwischen den CusS/ CusR and YedV/ YedW Zweikomponentensystemen.

Summary

Microorganisms are faced with a huge variety of environmental conditions. They use two-component signaling systems to sense and adjust their metabolism accordingly. Such two-component systems are highly abundant in genomes of bacteria, archaea and lower eukaryotes. They are comprised of a sensor histidine kinase and a response regulator which serves as a transcription factor regulating gene expression. Induction of two-component system signaling leads to autophosphorylation of the histidine kinase and the subsequent transfer of the phosphoryl-group to the response regulator. Both modularity and protein sequence similarities can give rise to signal integration between two-component systems at different levels of their signaling pathways. While cross-talk between non-cognate components of two-component systems has been assessed *in vitro*, not much is yet known about cross-talk *in vivo*. In this work, we identified *in vivo* interactions of two-component systems in the enterobacterium *Escherichia coli* using FRET microscopy. We have also characterized physiological relevant interactions of non-cognate components of the CusS/ CusR and YedV/ YedW two-component systems. In addition, our studies with promoter reporters and transcriptomic analysis showed two interconnection mechanisms, one between the histidine kinases and the non-cognate response regulators, the other on the level of induction of gene expression. Our data provide a detailed understanding of this cooperative regulation of target genes and cross-talk between the CusS/ CusR and YedV/ YedW two-component systems.

1 Introduction

This work is focused on studying signal transduction in two-component systems (TCSs) of the Gram-negative model microorganism *Escherichia coli* [1]. We are interested in how the activation of signal transduction of the 30 *E. coli* TCSs in response to environmental stimuli gets integrated into a particular cell response [2]. Here we focus on cross-talk among different TCSs. The cross-talk among TCSs associated with the response to an extracellular copper stimulus exemplifies how cells regulate the integration of signals in order to respond to pressing conditions.

1.1 Signaling in bacteria

Prokaryotes are ubiquitous in a wide range of environments on earth which challenge them for instance with fluctuations in temperature, oxygen and nutrient availability. To alter their metabolism, lifestyle or response to stress and external signals, bacteria regulate differential gene expression via signal transduction. External signals can be small molecules which enter the cell and function as effectors, but in many cases the signal is detected by a sensor which transmits to the regulatory machinery of the cell [3]. One-component systems have a sensor input and an output domain in one protein. They make up a vast part of intracellular prokaryotic signaling systems [4]. In eukaryotes signal transduction is predominantly mediated by Ser/Thr/Tyr kinases. They are less abundant in prokaryotes where the second most important signal transduction pathway are two-component systems (TCSs). Both systems rely on phosphoryl-transfer as a means of signal transduction [5], [2]. Other intracellular signaling systems utilize small molecules like cyclic-di-GMP, guanosine tetraphosphate (ppGpp) or cyclic adenosine monophosphate (cAMP). It has also been shown that the uptake and phosphorylation of sugars by the phosphotransferase systems (PTS) impacts global regulators as well as small regulatory RNAs, which are for instance involved in bacterial quorum sensing and can play a role in the regulation of gene expression in response to stimuli [6],[7]. The unique feature of TCSs among the described signaling systems is the extracellular or

periplasmic sensing whereas the others require the stimulus to enter the cell before detection.

1.2 Two-component systems

The wide range of signal recognition and regulation of physiological processes, cell division and virulence by TCSs makes them an interesting subject when studying signaling networks in bacteria. After one-component systems TCSs are the second most predominant signaling system in bacteria [2]. They are also involved in the signaling of archaea and eukaryotes with the exception of the animal kingdom where no genes encoding for TCSs have been found yet [8]. The number of TCSs genes in bacterial genomes can vary greatly and is correlated with the lifestyle and the environmental conditions the cell faces. The genomes of pathogenic bacteria encode for only a few or even no TCSs whereas bacteria faced with challenging environments can have even more than hundred TCSs (*Mycoplasma hypopneumoniae*: 0; *E. coli* MG1655: 30; *B. subtilis*: 32; *Anaeromyxobacter dehalogenans 2CP-1*: 76) [2].
TCSs are comprised of two proteins a sensor histidine kinase (HK) and a response regulator (RR) (Figure 1.2.1). In a prototypical, so called orthodox, TCS stimuli are detected by the cytoplasmic membrane-bound HK. As a result the HK forms a homodimer which catalyses the activation of the HK by ATP hydrolysis and phosphorylation of a conserved histidine residue. The phosphoryl-group is subsequently transferred to the RR which is located in the cytoplasm. The bonding of the phosphoryl-group to a conserved Asp residue in the RR triggers dimerization and a conformational change in the protein. The activated RR binds to DNA as a transcription factor (TF) which promotes transcription initiation of the downstream genes.

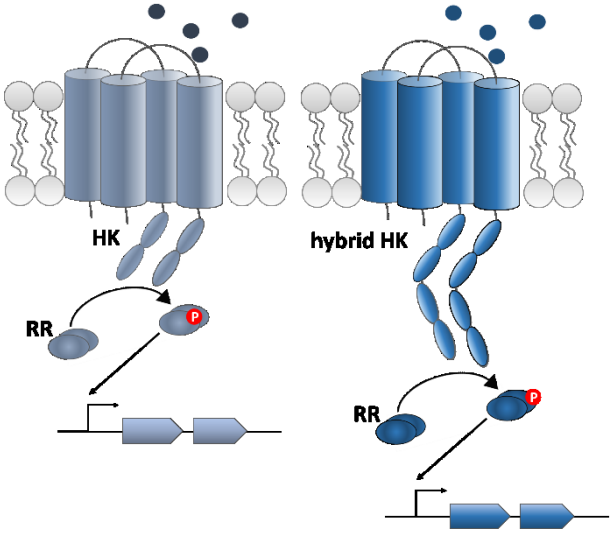

Figure 1.2.1 Schematic of two-component system (TCSs) signaling pathways.
The pathway consists of cytoplasmic membrane- bound sensor histidine kinase (HK) or hybrid HK dimer and a response regulator (RR) dimer in the cytoplasm. A periplasmic stimulus (blue circles) is detected by the HK or hybrid HK which then phosphorylates the RR (in the hybrid HK with additional phosphotransfer steps in between). The binding of the RR to promoter regions on the genome activates the expression of target genes.

Variants of orthodox TCSs are so called phosphorelays. They include more domains and phosphoryl-group transfer steps. In *E. coli* 5 out of 30 TCSs are phosphorelays with the hybrid HKs RcsC, TorS, ArcB, EvgS and BarA they are involved in cell surface remodelling [9], trimethylamine metabolism [10], respiratory and fermentative metabolism [11], resistance to variable drugs [12] and are indicated to be involved in pilus adherence and virulence [13]. A schematic of a phosphorelay can be found in Figure 1.2.1. Together with orthodox TCSs, they are involved in regulating anaerobic gene expression, different metabolisms, resistance to diverse toxic compounds, osmotic pressure, and many more functions in *E. coli* [5]. An overview of TCSs and their function is depicted in Table 1.2.1.

Table 1.2.1 TCSs in *E. coli*

TCS (HK/ RR)	Stimulus	Involved in	Reference
ArcB/ ArcA	oxidized quinone (negative stimulus)	repression of genes involved in respiratory metabolism	[11]
AtoS/ AtoC	acetoacetate	short-chain fatty acid metabolism	[14], [15]
BaeS/ BaeR	envelope stress	multidrug resistance	[16], [17], [18]–[20]
BarA/ UvrY	formate, acetate, propionate	Control of carbohydrate metabolism	[21]
BasS/ BasR	excess iron	modification of lipopolysaccharides	[22]
CheA/ CheB & CheY	aspartate, serin	chemotaxis	[23]
CitA/ CitB	citrate	citrate fermentation	[24]
CpxA/ CpxR	alkaline pH, high salt, metals, misfolded proteins	regulation of folding factors, proteases and surface structures	[25], [26]
CreC/ CreB	n.d.	regulator of metabolism	[27]
CusS/ CusR	copper	resistance to high copper concentrations	[28], [29]
DcuS/ DcuR	C4-dicarboxylates (e.g. fumarate)	response to C_4-dicarboxylates	[30], [31], [32]
EnvZ/ OmpR	osmolarity changes	modulation of outer membrane porins	[33]
EvgS/ EvgA	acidic pH, alkali metals	acid resistance	[34]
HydH (=ZraS)/ HydG (=ZraR)	zinc, lead	resistance to zinc and lead	[35]
KdpD/ KdpE	K^+ limitation, high osmolarity	regulation of K^+ transporters	[36], [37]
NarQ/ NarP	nitrate, nitrite	expression of genes involved in anaerobic respiration	[38], [39]
NarX/ NarL	nitrate	expression of genes involved in anaerobic respiration	[38], [39]

TCS (HK/ RR)	Stimulus	Involved in	Reference
NtrB (=GlnL)/ NtrC(=GlnG)	2-ketoglutarate, glutamine	survival under nitrogen limited growth conditions	[40], [41]
PhoQ/ PhoP	Mg^{2+} limitation	virulence, adaptation to Mg^{2+} limitation	[42], [43]
PhoR/ PhoB	P_i (negative stimulus)	phosphate homeostasis	[44], [45], [46]
QseC/ QseB	autoinducer-2	quorum sensing, flagellar biosynthesis	[47]
RcsC/ RcsB (=YojN)	osmotic shock, chlorpromazine, and others	motility	[48]
RstB/ RstA	acidic conditions	acid tolerance, curli fimbria formation, anaerobic respiration	[49], [50]
TorS/ TorR	TMAO	alkaline stress defence	[10], [51]
UhpB/ UhpA	glucose-6-phosphate	sugar transport	[52]
YedV/ YedW	H_2O_2	reduction of oxidative damage	[53]
YehU (=LytS)/ YehT (LytR)	n.d.	stationary-phase control	[54]
YfhK/ YfhA	n.d.	n.d.	n.d.
YpdA/ YpdB	extracellular pyruvate	nutrient utilization before entry into stationary phase	[55]
-/ FimZ (=YbcA)	n.d.	fimbrial expression, drug resistance	[56]
-/ RssB (=SprE)	n.d.	σ^S degradation, mRNA stability	[57], [58]

1.3 TCS structure and signaling mechanisms

1.3.1 Histidine kinases

Stimuli and changes in environmental conditions are sensed by HKs which transform them into a signal by catalysing auto-phosphorylation and activation of a RR. The domain organization of most HKs is an N-terminal input sensor domain which is located in the cytoplasm (in Gram-negative bacteria) or periplasm (in Gram-positive bacteria) between two transmembrane helices (TMHs) and a C-terminal cytoplasmic

transmitter domain which forms the catalytic core of the HK [59]. The general domain organisation is depicted in Figure 1.3.1.

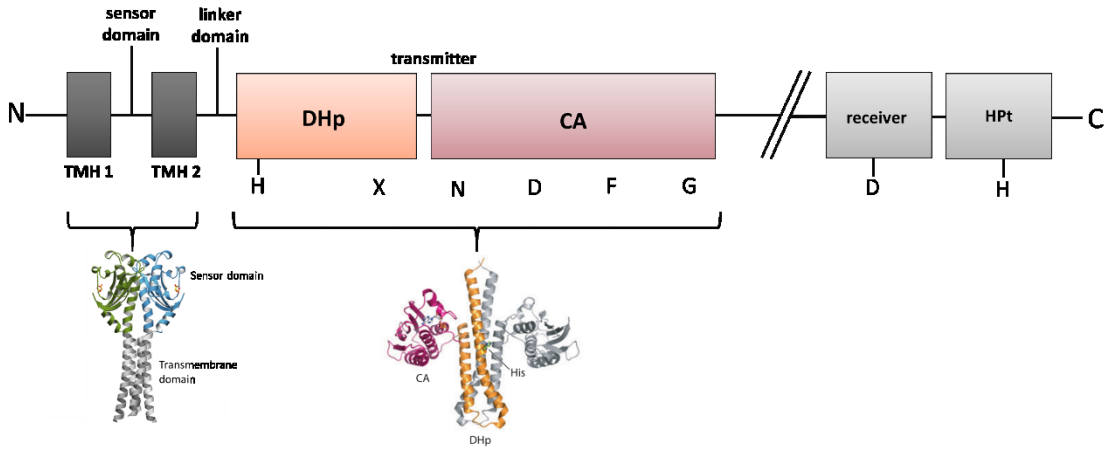

Figure 1.3.1 General histidine kinase domain organisation
The N-terminal part of the HK is formed by transmembrane domains (TMHs) and a periplasmic sensor domain. The linker and transmitter domains form the cytoplasmic part of the HK and contain conserved residues. Additional C-terminal domains (receiver and HPt) are part of hybrid HKs in a phosphorelay (modified after [59]). Crystal structure model of a HK's sensor and transmembrane domain of *E. coli* DcuS [60] and the transmitter domain of HK852 from *Thermotoga maritima* [61]

The sensor domain of a HK is responsible for the detection of the stimulus. It has a highly variable structure and sequence which enables HKs to sense a large variety of stimuli. The sensor domain in prototypical HKs is located in an extracytoplasmic loop, but HKs can also have sensor domains within the membrane or in the cytoplasm. The location of the stimulus perception is the feature that is used to classify HKs into three groups. The grouping takes the protein sequence and the domain architecture into account. Group I is the largest group and contains HKs with two TMHs which are typically sensing periplasmic solutes or nutrients [60]. A more detailed domain organisation of a TCS with a Group I HK/ hybrid HK is depicted in Figure 1.3.2.
Group II possesses up to 20 TMHs but no prominent periplasmic sensor domain. HKs of Group II mostly sense stimuli associated with membrane alterations caused by e.g. changes in temperature (DesK of *B. subtilis* [62]), redox potential (SenS of *Streptomyces reticuli* [63]), transport mechanisms or by interaction with membrane-

bound proteins. Members of Group III sense cytoplasmic stimuli and possess either a membrane anchor or are soluble in the cytoplasm. As over 80 % of HKs possess TMHs soluble HKs are not that common in bacteria [64].
The sensor domain of HKs of Group I can be comprised of mixed alpha-beta folds, all-alpha folds or folds which are similar to periplasmic binding proteins. A prevalent mixed alpha-beta fold motive is called PDC (PhoQ-DcusS-CitA) domain which is similar to the topology of PAS domains, a widespread motif in signal transduction proteins [60], [65]. Not much is known about how HKs of Group II sense stimuli. Hypotheses propose that either the TMHs form a hydrophobic compartment in the membrane where the lipophilic stimulus can interact or the TMHs probe the membrane for changes in its physical or chemical parameters [59].
Cytoplasmic sensor domains can be found in two locations: either at the N-terminus of the protein or between the last TMH and the catalytic core of the HK. Typical motifs are PAS, GAF or tripartite PCD folds which contains both PAS, GAF and an additional phytochrome domain [65]–[67], [68].

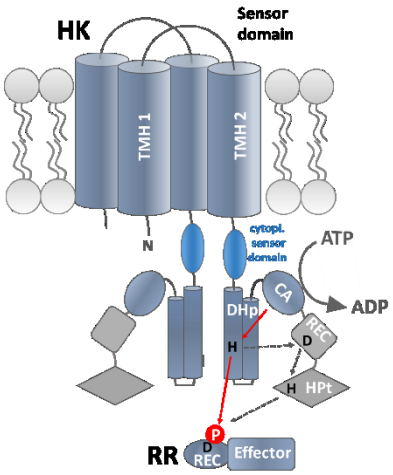

Figure 1.3.2 Detailed structure of a TCS with orthodox HK or hybrid HKs and phosphoryl-group transfer steps within the molecules
blue: orthodox HK and RR; light blue: additional cytoplasmic sensor domain; grey: additional domains of hybrid HKs.
Figure modified after [26]

The cytoplasmic part of the HK consists of a C-terminal DHp (dimerization and histidine phosphotransfer) domain (also known as: HisKA domain) and an N-terminal catalytic ATP binding (CA) domain (also known as: HATPase domain).
The DHp domain contains a conserved phosphoryl-group accepting histidine (His) residue and a structural two alpha-helix motif called the X box. [59]. The conserved His is a common feature for all HKs. The cytoplasmic domains of the *E. coli* EnvZ cytoplasmic domain shows a four-helix bundle comprised of two DHp domains [69]. DHp domains therefore play an important role in the dimerization of the HK monomers. The DHp domain is connected to the CA domain through a linker of variable length and sequence. This linker is implicated to be involved in the movement of the CA domain in relation to the DHp domain. This movement is required for the auto-phosphorylation of the HK [62], [70].
The CA domain belongs to the ATPase/kinase GHKL superfamily and catalyses the hydrolysis of ATP [71]. Conserved residues of the CA domain form an ATP-binding fold first described by Bergerat and colleagues [72]. The domain structure is split into N, D, F and G boxes. Each box bears characteristic conserved residues involved in the hydrolysis of ATP. The N box contains an asparagine (Asn) residue which coordinates an Mg^{2+} ion. This ion connects all phosphates of the ATP to the HK via hydrogen bonds. The D box's aspartic acid (Asp) residue is responsible for the specificity to ATP. A lid typical of the Bergerat fold is formed by the G box which is further supported by a box specific for HKs containing phenylalanine (Phe) and tyrosine (Tyr) residues (F box). The binding of the triphosphate nucleotide (ATP) stabilizes the ATP-lid. It destabilizes after the hydrolysis due to the formation of the diphosphate (ADP), and becomes stabilized again by the interaction with a RR [70], [73]–[75]. Upon binding of the ATP the CA domain rotates towards the conserved His residue of the DHp domain. The His residue reacts with the γ-phosphate of the ATP via nucleophilic attack yielding His~P and ADP. The phosphorylation of the His residue can to happen both in *trans* (between the subunits of the homodimer) and in *cis* (within a monomer). Examples for both have been found in *E. coli* HKs (*trans*: NtrB and EnvZ [76], [77], *cis*: ArcB [78]). Furthermore the phosphorylation results in a rearrangement of the DHp domain helices which presents the HK's docking site to the RR. This reaction is required for the signal transduction to RR [62], [70].

Signals detected by the sensor domain of the HKs have to be transferred to the catalytic core and a small conformational change is thought to trigger the auto-phosphorylation of the HK. The signal is further transduced by a distortion of the sensor domain which is for instance caused by the binding of citrate in the CitA HK of *Klebsiella pneumoniae* [79]. It results in a piston-like sliding movement between the N- and C-terminal TMHs. A similar mechanism was found for the aspartate receptor Tar and for NarX in *E. coli* [80] [81]. The movement of the TMH is thought to activate the catalytic part of the HK for signaling. The mechanism to transduce the signal from the sensor to the catalytic domains can show slight differences among HKs but always results in an activation of the cytoplasmic part of the respective HK. [60].
The activation of the HKs can be summarized as a process of stimulus sensing followed by signal transduction over the membrane. It triggers the movement of the ATP binding CA domain towards the phosphoryl-group accepting His residue in the N-terminal DHp domain. Additional domains like a RR-like receiver (REC) domain and an HPt (histidine-containing phosphotransfer) domain can be part of hybrid HKs in a phosphorelay and introduce two additional phosphoryl-group transfer steps into the HK signaling. Orthodox HKs subsequently transfer the phosphoryl-group from the conserved His residue to the receiver (REC) domain of the RR.

1.3.2 Response regulators

RRs are phosphorylation-activated switches which are able to bind DNA. They bind to tandem or inverted repeats in the promoter region of target genes and serve as transcription factors (TFs) by recruiting the RNA polymerase to this region to initiate transcription [82]–[85]. They are comprised of an N-terminal receiver (REC) domain which interacts with the HKs and accepts the phosphoryl-group and a C-terminal effector domain which in most RRs is responsible for interactions with the DNA. The RR interacts with the DHp domain of the active HK. A conserved Asp residue in the REC domain forms bonds with the His~P and in a nucleophilic substitution reaction the bond between the His residue and the phosphoryl-group is broken while a new bond to a conserved Asp residue in the RR is formed [70]. It is proposed that this reaction is mainly catalysed by the RR which is thought to pull the phosphoryl-group away from the HK. Some RRs can also auto-phosphorylate using small molecules such as acetyl

phosphate, carbamyl phosphate, and others [86], [87]. For both reactions an Mg^{2+} ion bound to Asp residues in the phosphoryl-group accepting pocket of the REC domain is required.
The C-terminal effector domain is responsible for the binding of the RR to the DNA. In *E. coli* 25 of 32 RRs have DNA binding domains. In total about 65 % of the RRs possess a DNA binding domain, others are for instance involved in phosphoryl-shuttling and in interactions to regulate the flagellar motor [88]. The structure of the effector domain is used to group RRs into three major families. The OmpR family represents one-third of all RRs. The typical feature is a winged-helix motif for contact with the DNA. About 50 % of the members of the OmpR family form homodimers upon activation. Dimerization is required for their output response and is mediated by conserved residues in the REC domain [89], [90]. The NarL family RRs bind the DNA with a helix-turn-helix motif. The third NtrC family has an ATPase domain followed by a helix-turn-helix DNA binding domain. The ATPase activity requires an oligomerization of the domains and is controlled by the receiver domain [88]. There are also RRs which have a C-terminal domain with enzymatic or mechanistic activity [88]. One example is CheB which functions as methylesterase in the chemotaxis pathway [23]. Other RRs even lack effector domains and their function is not clear yet [88].
An example how the RR activation takes place is the *E. coli* RR NarL. In the inactive state the DNA recognition site is turned towards the receiver domain and a slight repositioning of domains by phosphoryl-group binding is necessary for letting the effector domain interact with the DNA [91]. This is also possible because the phosphorylated RR has a higher affinity for target promoters than the unphosphorylated RR [92]. The binding initiates the recruitment of the RNA polymerase and the transcription of target genes [93].

1.3.3 Hybrid HKs and phosphorelays

Phosphorelays are more complex versions of the TCS signaling pathway and referred to as unorthodox TCS. In addition to the HK's DHp domain and the-RR's REC domain a phosphorelays possess additional phosphoryl-group accepting domains. A domain similar to the receiver domain of a RR is followed by an HPt domain. The additional receiver domain has a conserved Asp residue and accepts the phosphoryl-group from

the DHp domain of the hybrid HK. The HPt domains consist of about 120 amino acids and has a short consensus motif which includes a conserved His residue. It accepts the phosphoryl-group from the additional receiver domain and transfers it to the RR's Asp [94]. With the additional domains the phosphotransfer scheme is His-Asp-His-Asp as shown in Figure 1.3.2.

The first three phosphoryl-group accepting residues can either be part of one or part of three independent proteins. In *Bordetella pertussis* BvgS is part of a TCS which activates the transcription of virulence factors. BvgS is comprised of three cytoplasmic domains and is an example for a multidomain protein phosphorelay [95]. Multidomain proteins like BvgS are referred to as hybrid HKs (also hybrid-type HKs) and can make up to 25 % of all HKs in a bacteria's genome [96].

Phosphorelays with separate proteins are typical of eukaryotes like yeast and *Arabidopsis thaliana* [97]–[99]. There are indications suggesting that the hybrid HKs in eukaryotes were acquired from bacteria through lateral gene transfer [100]. In prokaryotes phosphorelays can have a hybrid HK with an independent HPt domain or such as *B. subtilis* possess two independent proteins. In the sporulation pathway of *B. subtilis* Spo0F (regulatory domain) and Spo0B (phosphotransfer domain) introduce additional steps between the HK and the RR Spo0A [101]. The RcsC-YojN-RcsB signaling pathway in *E. coli* is involved in the capsular synthesis and swarming. It is an example for a hybrid HK (RcsC) with an independent HPt domain (YojN). However YojN lacks a phosphorylation site and therefore its function is not clear yet [94]. Other hybrid HKs in *E. coli* are TorS, ArcB, EvgS and BarA. All four are hybrid HKs with an additional receiver and an HPt domain [94], [102].

Interestingly phylogenetic analysis suggest that there is no common ancestor for hybrid kinases. Most likely they arose from lateral recruitment of receiver domains into the HK molecule and duplication as one unit. Therefore their distribution among closely related microorganisms and their combinations of individual kinase and receiver domains varies strongly [96].

1.3.4 Pathway specificity and pathway variants

The signaling pathway of TCS can be described as linear signaling between a HK or hybrid HK and a RR resulting in the activation/ repression of target gene transcription. Bacteria can have up to over hundred TCSs which all share ~~as~~ high degree of sequence

and structural conservation in the subdomains. High similarity can give rise to interaction and phosphotransfer between non-cognate components. Indeed it has been shown that HKs phosphorylate non-cognate response RRs *in vitro*. An extensive screen of *E. coli* HKs and RRs performed by Yamamoto and colleagues showed transphosphorylation for 22 non-cognate HK-RR combinations out of 692 tested pairs [103] as did a similar study by Skerker and colleagues for several HKs from *E. coli* and *Caulobacter crescentus*. Transphosphorylation of non-cognate RRs by *E. coli* EnvZ, CheA and CpxA as well as CC1181 from *C. crescentus* was outcompeted by the phosphorylation of the cognate RR which happened within seconds [104]. The phosphorylation rates of non-cognate pairs are generally much slower than between cognate pairs. The same holds true for heterologous phosphorylation between the vancomycin resistance TCS VanSR of *Enterococcus faecium* and the PhoB RR of *E. coli* which exhibited a 30-fold higher binding preference for the cognate components [105], [106]. High sequence similarity (e.g. >35 % for VanR and PhoB) shows that the distinction between non-cognate and cognate partner is not conveyed by striking differences in the protein structure. In fact several 'anchor' residues which are necessary to fit HK and RR molecules together have been identified in the DHp domain of HKs. The domain also contains variable residues that prevent productive interaction of 'anchor' residues between non-cognate systems [107], [108]. An alteration of these coevolving residues can rewire the TCS's specificity [70], [109], [110]. Specificity of signaling is therefore controlled by the HK's intrinsic ability to recognize its cognate substrate with residues in the interaction surface and a quick phosphorylation reaction that excludes other RRs. This specificity is further promoted by the relative low abundance of HKs compared to RRs. In the well-studied *E. coli* TCS EnvZ-OmpR the ratio of the RR and its HK has been determined to be 35-fold higher and similar values have been found for other TCSs [33], [111]–[115]. It was also shown that the transcriptional output of the system is robust to a wide range of expression levels of both HK and RR which is also a general feature of TCSs [112].

Many TCSs have an additional property to control specificity. Without a dephosphorylation step the RRs' half-lives can be up to several hours and thereby cause unwanted signaling. Phosphatases can trigger the RR to dephosphorylate and convert it to an inactive state [116], [117]. The phosphatase activity can either be exercised by an

independent protein or by bifunctional HKs. In addition to the typical ATPase activity they are also able to dephosphorylate their cognate RR to shut down unwanted activation for instance by a non-cognate HK [111], [118]–[120], [121]. In this reaction the cytoplasmic domain of the HK is likely to catalyse a nucleophilic attack of the high energy Asp~P bond by a water molecule [70], [122] [123]. Bifunctional HKs are able to suppress unwanted phosphorylation more efficiently than monofunctional HKs [111].
Even though TCSs insulate their signaling, several variants expand the linear signaling to more elaborate branched pathways (Figure 1.4).

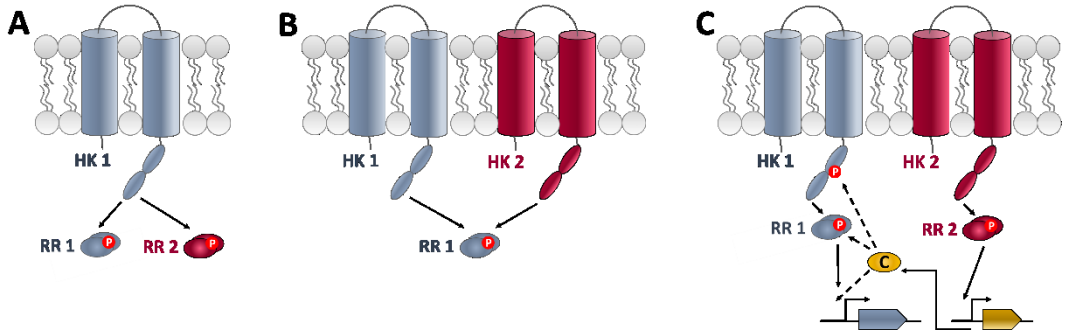

Figure 1.3.3 Branched-pathway architecture
(A) 'one-to-many' pathway with several RRs being phosphorylated by one HK; (B) 'many-to-one' pathway with several HKs phosphorylating one RR; (C) pathway with connector protein which establish a regulatory link between two independent pathways. Figure modified after [124]

The 'one-to-many' describes a pathway structure where a single HK phosphorylates more than one target RR (Figure 1.3.3 A). This can be observed for the *E. coli* HK ArcB which has been shown to phosphorylate the two RRs ArcA and RssB which control the σ^S level in the cell [57]. It can also be seen for the chemotaxis kinase CheA which phosphorylates the RRs CheY and CheB [23], [125].
Another case where many HKs regulate one RR is referred to as 'many-to-one' (Figure 1.3.3 B). In *B. subtilis* sporulation KinA, KinB , KinC and KinE phosphorylate the RR Spo0F [126]. Similarly quorum sensing in *Vibrio harveyi* is induced by three hybrid HKs. They phosphorylate the HPt protein LuxU which subsequently passes the phosphate on to the RR LuxO [7].
Connector proteins are able to influence TCSs by affecting them at several levels (Figure 1.3.3 C). They can interfere at the level of the HK where they can either block

its auto-phosphorylation or activate the HK [127], [87], [129]. In addition to promoting or inhibiting the dephosphorylation of RRs, connectors can interfere with the interaction of RRs and DNA as well as with its ability to recruit RNA polymerase to start transcription [130]–[134]. Connectors often establish regulatory links between independent TCS signaling pathways. In *Salmonella enterica* the connector protein PmrD connects the two TCSs PhoP/PhoQ and PmrA/PmrB and causes PmrA-dependent gene expression under PhoP/PhoQ inducing conditions [135]–[137]. In *E. coli* the EvgA/EvgS system activates the expression of the inner membrane protein B1500 which forms a complex with and activates the PhoQ HK [128]. Connectors can broaden the signal spectrum which can influence and promote the activation of a RR and therefore the signaling output of TCS signaling.

1.3.5 Cross-talk among two-component systems

Cross-talk can be described as a cross-interaction between two otherwise distinct signaling pathways. In contrast to detrimental cross-interaction which causes the abrogation of a signal it is advantageous to the cell [111], [138], [139]. As TCSs detect a huge variety of stimuli simultaneously, they are likely to work in parallel. As described above the domains organisation of TCSs is highly modular and therefore can give rise to cross-interactions. Cross-talk can provide a mechanism for processing multiple signals [118], [138]–[140]. It can take place on the level of HKs where instead of the interaction of cognate HKs a heterodimerization between non-cognate HKs can arise or a higher-order signaling complex can be formed. (Figure 1.3.4 A). Cross-phosphorylation between non-cognate HKs and RRs and the heterodimerization of non-cognate RRs are other means of signal integration (Fig 1.3.4 B and C). Finally cross-talk can happen on the promoter level where the expression of genes can be regulated by RRs which normally do not control them (Fig. 1.3.4 D)

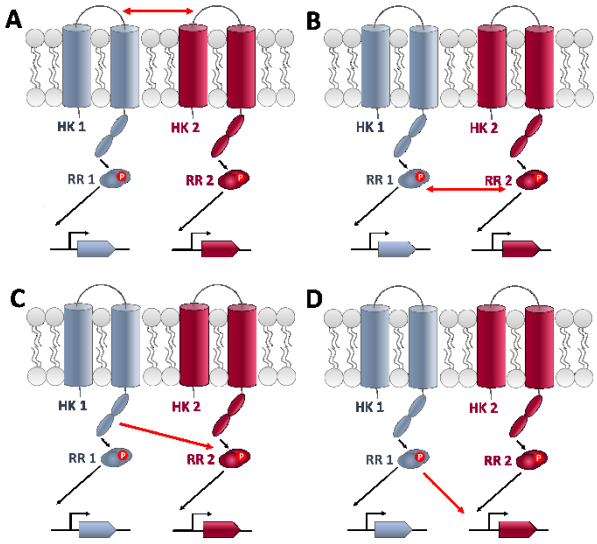

Figure 1.3.4 Levels of cross-talk between TCS pathways
(A) Heterodimerization or clustering of HKs resulting in cross-activation of the TCSs; (B) Cross-interactions between RRs by heterodimerization; (C) cross-phosphorylation of a non-cognate RR; (D) Initiation of gene expression by a non-cognate RR.

HKs have the ability to form clusters in the cell membrane. The chemotaxis receptors of *E. coli* forms large hexagonal complexes of receptors surrounded by the HK CheA and coupling proteins at the cell poles [141]–[143]. Clustering has also been reported for the HKs DcuS and CitA which accumulate at the cell poles [144] as well as for the TorS and EvgS HKs in *E. coli* [95]. Sensor clusters can amplify the response to a stimulus. In case of heterodimeric clusters multiple perceived stimuli can be integrated [146]–[150]. As described in Chapter 1.3.4 cross-talk from a HK to a non-cognate RR is a common phenomenon *in vitro*. This is supported by studies that found phosphorylation of non-cognate RRs *in vivo*. However, in many cases the observed *in vivo* cross-talk would take place only in the absence of cognate components like the HK [118], [151]–[154], [155] [115]. Nevertheless there are also cases of *in vivo* cross-talk which happen under native conditions [57] [23], [125]. A prominent example is the cross-talk between the TCSs NarX/ NarL and NarQ/ NarP which regulate the transcription of nitrate respiratory genes. Whereas the HK NarQ gets stimulated by both nitrite and nitrate the HK NarX only gets activated by nitrate. NarQ is not only promiscuous in the recognition of stimuli but also in the recognition of RRs. It can phosphorylate both NarL and NarP *in vivo* enabling a fine tuned response to high and low concentrations of nitrate [38],

[39]. There is also evidence for cross-talk between the ArcB/ ArcA and EnvZ/ OmpR TCSs in *E. coli* [156], the PhoR/ PhoP and YycG-YycF TCSs of *B. subtilis* [157], [158] and the HKs NtrB and NtrY to NtrC in *Rhodobacter capsulatus* [159].
The activation or repression of genes that are part of a TCS's regulon by non-cognate RRs has been reported for many systems. Gene promoters can possess binding sites for several different transcription factors. Promoter transcriptional fusions for instance showed regulation of expression of the periplasmic chaperon Spy by the BaeR and CpxR RRs in response to extracytoplasmic stress signaling in *E. coli* [160]. CpxR also regulates the Curli regulator CsgD together with the OmpR RR [161]. The identification of further examples for beneficial cross-talk among TCSs can increase our understanding of how the variety of stimuli gets integrated into the signaling network in bacteria.

1.4 Copper homeostasis and sensing

1.4.1 Copper homeostasis in bacterial cells

An example for an environmental stimulus recognized by many bacteria is copper, which on the one hand is an important enzyme cofactor but on the other hand can also cause damage to the bacterial cell. Copper became bioavailable when oxygen in the earth's atmosphere increased due to the development of oxygenic photosynthesis by Cyanobacteria around 2 billion years ago [162]. Before that time copper on earth was present as water-insoluble Cu(I) in sulphides. Oxidization to Cu(II) made it soluble [163]. In most bacterial habitats such as the intestinal tract or soil the available copper concentration is low. Anthropogenic input for instance from mining or agriculture can increase the heavy metal concentration in the environment [164], [165]. Modern soil might be more rich in copper due to an agricultural method which uses copper salts as feed additive and fungicide [166]. Nevertheless copper is important to the bacterial cells as the presence of oxygen also created the need for high redox-active metals with redox potentials between 0- 800 mV in the reaction centre of bacterial enzymes compared to the previously needed low redox potentials [162], [163]. Enzymes with copper in their reaction centre work between 250- 750 mV. Because of their high redox potential they are involved in oxygen transport and activation as well as electron transfer reactions

[167]. An example for an enzyme with copper cofactor is the bacterial cytochrome c oxidase, which is a member of the Heme-Cu respiratory oxidase family. These oxidases are responsible for the reduction of oxygen and other electron acceptors and creation of an H^+ gradient over the cytoplasmic membrane as part of the respiratory chain in bacteria [168], [169]. Other examples are the multicopper oxidase CueO which oxidizes periplasmic Cu(I) to Cu(II) [170] and the Cu, Zn superoxide dismutase (Cu, Zn-SOD) which oxidizes several substrates like NADPH in a HCO_3^-dependent manner [166]. An analysis of the metalloproteom of *Pyrococcus furiosus* and *E. coli* suggest that there are also many more unidentified metalloproteins, including copper-containing proteins, in bacterial genomes which have not been found and characterized yet [171].
Even though the redox potential of copper is ideally suited only around 1 % of the metal containing enzymes have a copper cofactor [172]. Despite the utility of copper in the enzymatic reaction centre of enzymes it also has a disadvantageous property: free copper is toxic to the cells. The redox activity of copper ion leads to the formation of reactive oxygen species (ROS). In a Fenton-like Haber-Weiss reaction, like it is known for iron, Cu(II) is reduced to Cu(I) in the presence of superoxide radicals (O_2^{-*}). In a second reaction Cu(I) is oxidized by hydrogen peroxide (H_2O_2) back to Cu(II) forming hydroxide ions (OH^-) and an extremely reactive hydroxyl radical (OH^*) [173], [174]. The resulting oxidative stress could lead to damage of cell structures but has been critically discussed recently as copper fails to cause oxidative damage to the DNA [175]. It has been proposed that an overload of copper in the cell leads to an acceleration of the iron-based Fenton reaction rather than a copper-based one [175].
Therefore the major role of copper toxicity seems to be played by non-oxidative mechanisms. Cu(I) which predominates in the reducing environment of the cell and under anaerobic conditions can displace other metal ions bound to thiolate or sulphide ligands [166], [176]. It can interfere with iron-sulfur clusters and their biogenesis by iron-sulfur cluster assembly systems [177], [178]. An example is the inactivation of the iron-sulfur cluster of isopropylmalate dehydratase, an enzyme involved in the biosynthesis of branched-chain amino acids, which causes a deficiency in the cell growth [177]. As enzymes containing iron-sulfur clusters make up around 5 % of all metalloproteins, the damage caused to them has a big impact on the cell's metabolism [179].

The mechanisms how copper reaches the cytoplasm where it exhibits its toxic potential are not well understood yet. In gram-negative bacteria copper seems to cross the outer membrane non-specifically through porins (e.g. OmpC), as a porin-deficient *E. coli* mutant was resistant to copper [180]. The copper in the periplasmic space crosses the cytoplasmic membrane in an unknown fashion. Bacteria lack high-affinity transport systems for copper like described in *Saccharomyces cerevisiae* and in other eukaryotes [181]. Therefore Cu(I) might enter the cell through non-specific uptake systems for other metals [176]. There are speculations about Cu(II) entering the cytoplasm via the Zn(II)-uptake system ZnpT as it has a broad substrate specificity [182]. Nevertheless the mechanism of how copper enters the prokaryotic cells still remains to be resolved in detail. As there seems to be no mechanism controlling the influx of copper into the cell, the efflux plays a major role in determining the intracellular copper level and sensing of copper is a prerequisite to control the expression of systems involved in it.

1.4.2 Copper sensing in *E. coli*

Intracellular copper is needed but also has to be kept at a low level to avoid detrimental side effects. The detection of copper levels is a prerequisite to implement further steps dealing with excess copper. Enterobacteria like *E. coli* possess two genome-encoded systems dealing with copper. Some strains isolated from a high-copper environment additionally possess a plasmid-encoded system [167].

One of the genome-encoded systems is the one-component system CueR [176]. Cytoplasmic Cu(I) binds and activates CueR, a MerR-family transcription factor which controls the expression of CopA, a Cu(I)-translocating P-type ATPase in the cytoplasmic membrane [183], [184]. Cu-transporting P-type ATPases export copper unidirectional while hydrolysing ATP [185]. CopA shuttles Cu(I) from the cytoplasm to the periplasmic space. Additionally the Cu(II) present in the periplasm can be reduced to the more toxic Cu(I) by NADH dehydrogenase II and components of the respiratory chain. The re-oxidation of the accumulating Cu(I) depends on copper-dependent copper oxidases [182]. CueR also controls the expression of CueO a periplasmic multicopper oxidase which protects periplasmic enzymes from copper-induced damage by oxidizing Cu(I) to less toxic Cu(II). This oxidation step is coupled to the reduction of oxygen to water. CueO is an essential part of the cell's mechanism of dealing with excess copper

but its only functional under aerobic conditions [186], [187]. Therefore the lack of CueO functionality has to be compensated under anaerobic conditions.

E. coli possesses a second copper efflux system which is found exclusively in Gram-negative bacteria. The proteins CusC, CusB and CusA form an active transport channel which is using proton motive force to drive the export of copper [28]. It is traversing both membranes and is assumed to export Cu(I) from both cytoplasm and periplasmic space [188]. The CusCBA transport system is associated with a periplasmic metallochaperone called CusF. CusF may bind both Cu(I) and Cu(II) and delivers them to the CusCBA protein complex. It is not as essential for copper resistance like CusA and CusB but it plays a role in the detoxification of the periplasm as deletion mutants of CusF are more susceptible to copper [188]. The CusCBA transport system facilitates the copper export over the outer membrane under aerobic and anaerobic conditions. Under aerobic conditions it is a backup system for CopA and CueO and helps the CueR regulated system to deal with higher amounts of copper. The expression of the CusCBA transport system is regulated by the CusS/ CusR TCS which senses copper and silver ions in the periplasm [28], [28], [176], [29]. The CusS HK binds periplasmic copper, which leads to the dissociation of its two TMHs and the exposure of an interaction surface for dimer formation and activation of the HK [189]. The phosphoryl-group transfer to CusR activates the RR which serves as a transcription factor for the *cusCFBA* operon as well as the *cusRS* operon thereby activating a positive feedback loop typical for two-component systems [29]. The amount of copper necessary to initiate the CusS signaling is significantly higher than the amount of copper necessary to activate CueR [29]. Under anaerobic condition where Cu(I) prevails CusS/ CusR is the only regulatory system dealing with excess copper in cytoplasm and periplasm whereas under aerobic conditions both the CusS/ CusR and the CueR systems are functional.

The CusR regulon was determined by transcriptional analyses, DNA microarray and DNaseI footprinting [28], [29]. CusR was found to bind upstream of the *cusRS* and *cusCFBA* operons and to regulate their transcription in a copper-dependent manner [29]. Interestingly the DNA microarray also revealed an increased expression of the genes *yedW* and *yedV*. YedV/ YedW is a putative TCS with unknown function. It is encoded in in the *yedWV* operon, described by Yamamoto and colleagues as *yedVW* operon. They also found that the copper-induced expression of the HK YedV was abrogated in

cusR-null mutants [29]. YedV and CusS are closely related HKs [110] and the YedV HKs has been shown to cross-phosphorylate the CusR RR *in vitro* [103]. These findings indicate a possibility for cross-interaction of the CusS/ CusR and YedV/ YedW system *in vivo*. A recent study suggests an overlap in the regulon of the two TCSs [53]. An afresh assessment of the CusR RR binding sites identified the intergenic region of *yedW* and *hiuH* (renamed from *yedX*) in addition to the already identified binding site in the intergenic region of *cusR* and *cusC*, which was thought to be unique before [29]. YedW was found to bind to the same two intergenic regions and additionally upstream of the *cyoA* gene. Expression of the *hiuH* gene was found to be induced either by copper-sensing through CusS/ CusR (detected at the level of protein expression) or by sensing of H_2O_2 by YedV/ YedW (detected at the level RNA) [53]. HiuH is a 5-hydroxyisourate hydrolase, a protein which is involved in the purine catabolic pathway of *B. subtilis* and *Salmonella* where it catalyses the conversion of 5-hydroxyisourate (5-HIU) to 2-oxo-hydroxy-4-caroxy-5-ureidoimadeazline [190], [191]. In *E. coli* this reaction might reduce oxidative damage by spontaneous 5-HIU degradation [53]. A cooperative regulation of *hiuH* and the other genes of the described regulon would be similar to the NarX/ NarL and NarQ/ NarP TCSs which are a good example for beneficial *in vivo* cross-talk [38], [39].

1.5 Aims of this work

TCSs play an important role in how prokaryotes perceive their environment and they have first been described as signaling pathways 30 years ago [192]. The huge variety of different TCSs in prokaryotic genomes has been researched ever since as they are involved in the regulation of a wide range of physiological processes. Though the structural organization of TCSs is relatively well understood, to date not much is known about interconnection of TCSs which regulate different physiological processes and how the signaling is integrated into an overall cell response. To address the role of interconnection and to elucidate the importance and the extend of cross-talk between TCSs in the enterobacterium *E. coli* we studied component interaction *in vivo*, signaling pathway activity and the influence of a TCS on the transcriptome of the bacterial cell. Ensuing interaction studies between fluorescent protein fusions of HKs and RRs to

identify interactions between both cognate and non-cognate components we investigated signaling and cross-talk between the TCSs YedV/ YedW, CusS/ CusR and, to a smaller extend, BaeS/ BaeR in more detail. These systems show indications for interconnection due to a shared HK self-interaction upon stimulation with copper. We wanted to explore and describe this potential for cross-regulation further.

2 Materials and Methods

2.1 Chemicals and consumables

A list of all chemicals and consumables used in this study is listed in Table 7.1.1 in the Appendix.

2.1.1 Media and plates

Minimal A Stock solution (5x)	5 g	$(NH_4)SO_4$
	22.5 g	KH_2PO_4
	52.5 g	K_2HPO_4
	2.5 g	Sodium citrate x 2 H_2O

ddH_2O was added up to a total volume of 1 L

Minimal A medium	200 µL	Minimal A stock solution
	1 mL	1 M $MgSO_4$ x 7 H_2O
	10 mL	20 % Glycerol
	5 mL	20 % Casamino acids

ddH_2O was added up to a total volume of 1 L

Luria broth (LB) medium	10 g	Bacto tryptone
	5 g	Bacto yeast extract
	5 g	NaCl

Adjusted with NaOH to pH 7 and ddH_2O was added up to a total volume of 1 L.

Tryptone broth (TB) medium	10 g	Bacto tryptone
	5 g	NaCl

Adjusted with NaOH to pH 7 and ddH_2O was added up to a total volume of 1 L.

Luria broth (LB) plates	1.5 g Agar/ 100 mL LB medium

2.1.2 Buffers and stock solutions

Phosphate buffer	1.742 g	K_2HPO_4
	1.361 g	KH_2PO_4
	0.901 g	lactic acid

Adjusted with NaOH to pH 7 and ddH_2O was added up to a total volume of 1 L.

TAE buffer (50x) for gel electrophoresis	242 g	Tris base
	57.1 g	Glacial acetic acid
	100 mL	0.5M EDTA (pH 8)

ddH_2O was added up to a total volume of 1 L.

TN buffer	20 mM	Tris-HCl pH 8
	0.2 M	NaCl

Antibiotic solutions	Ampicillin (Amp)	100 mg/mL in ddH_2O
	Chloramphenicol (Cam)	34 mg/mL in ethanol
	Kanamycin (Kan)	50 mg/mL in ddH_2O

Inducer solutions	0.1 M IPTG	in ddH_2O
	10 % L-Arabinose	in ddH_2O

2.1.3 Reaction Kits

- GeneJET DNA Purification Kit, ThermoFisher Scientific, Dreieich
- GeneJET Gel Extraction Kit, ThermoFisher Scientific, Dreieich
- GeneJET Plasmid Miniprep Kit, ThermoFisher Scientific, Dreieich
- Q5 Site-Directed Mutagensis Kit, New England BioLabs GmbH, Frankfurt a.M.
- EUR_X GeneMATRIX Universal RNA Purification Kit, Roboklon GmbH, Berlin
- TURBO DNA-free Kit, Ambion ThermoFisher Scientific, Dreieich

The kits were used according to the guidelines given by the manufacturers.

2.2 Strains

The strains used in this study are shown in Table 7.1.2 in the Appendix. All strains of this study are derived from the *E. coli* K-12 strains MG1655 [1] and BW25113 [193], the wildtype of the Keio collection [194].

2.2.1 Gene deletion strains derived from Keio collection

The kanamycin resistant single-gene deletion strains of the Keio collection were used as donor strains for P1 phage transduction [195] into the MG1655 background. The resulting strains were tested for correct insertion of the FRT-site flanked kanamycin cassette into the genome using gene and kanamycin cassette specific primers [193]. The kanamycin cassette was removed from the deletion strains using the temperature sensitive pCP20 plasmid encoding a FLP recombinase [196] and tested for the loss of antibiotic resistance after several rounds of growth on LB plates at 42°C. The resulting strains carry a 82-85 nucleotide scar in place of the disrupted gene [193] and are henceforth referred to as Δ*name of deleted gene* strains with $_{MG}$ or $_{BW}$ dependent on the wildtype strain background.

2.2.2 Deletion strains derived from genomic integration of PCR products

In order to create a desired gene deletion strain homologous recombination of a linear PCR product with the genomic DNA performed using a phage λ red recombinase [193]. The PCR product sequence contained a kanamycin resistance cassette flanked by FRT sites as well as homologous gene regions up and downstream of the integration site. Competent cells with λ red recombinase expressed from a plasmid (pDK46) were transformed with the linear DNA, incubated at 37 °C first in LB medium then on a kanamycin plate over night. To cure the cells from the temperature sensitive λ red recombinase plasmid 2-3 rounds of incubation at 42 °C on a plate over night were performed. Single colonies were tested for the loss of the ampicillin resistance on an ampicillin plate. The integration of the kanamycin resistance cassette into the desired locus of the genome a PCR amplification was performed using a kanamycin cassette/ gene specific primer set. The knockout was additionally confirmed with sequencing of a PCR product covering the entire genetic region. The kanamycin resistance cassette was then removed like described in Chapter 2.2.1.

2.3 Plasmids

Table 7.1.3 in the Appendix shows the plasmids used in this study.
pVS, pDK, pES, pAM and pAE plasmids were designed and cloned by Victor Sourjik, David Kentner, Erik Sommer, Anette Müller and Andreas Ernst. These plasmids were taken from the lab stocks. The pKD13 derivate used for the amplification of the FRT-site flanked *kan*^{r} gene was a gift by Juliane Winkler (AG Bukau, ZMBH Heidelberg) and taken from the lab stocks. Plasmids containing promoter GFP fusions were taken from an *E. coli* promoter collection [197].
Several pMF plasmid constructs used the modified pTrc99a plasmid pDK112. pDK112 is used for creating C-terminal YFP tagged fusion proteins. It contains a modified RBS as well as a part of the *cheB* gene fused to a monomeric eyfp^{A206K}. The modified RBS is flanked by *Spe*I and *Nco*I restriction sites, the eyfp^{A206K} is flanked by *BamH*I and *Hind*III restriction sites. In this study *Nco*I and *Hind*III were used to cut the *cheB(1—134)-GTG_YFP* fragment from the plasmid and to insert the desired gene sequence into the plasmid backbone. The *Spe*I and *Hind*III restriction enzymes were used to transfer the gene plus the modified RBS into the pBAD33 expression plasmid.

2.4 Cloning strategies

2.4.1 Primers

The list of primers used in this study can be found in Table 7.1.4 in the Appendix.

2.4.2 Polymerase Chain Reaction (PCR)

Single Colony PCR	25 µL	DreamTaq Green PCR Master Mix (2x)
	1 µL	forward primer (10 pmol/ µL)
	1 µL	reverse primer (10 pmol/ µL)
		colony picked from plate
	up to 50 µL	ddH_2O
Thermocycler settings	95 °C	3 min
	95 °C	30 sec

	55 °C	30 sec
	72 °C	variable (1 min/ 1 kb)
	72 °C	5 min
PCR with Q5 polymerase	10 µL	Q5 reaction buffer
	1.25 µL	forward primer (10 pmol/ µL)
	1.25 µL	reverse primer (10 pmol/ µL)
	1 µL	dNTPs (10 mM)
	0.5 µL	Q5 high fidelity DNA polymerase
	1 µL	template DNA
	up to 50 µL	ddH_2O
Thermocycler settings	98 °C	1 min
	98 °C	10 sec
	variable temperature	30 sec
	72 °C	variable (10 sec/ 1 kb)
	72 °C	2 min

The PCR reactions were performed in the thermocyclers TPersonal (Biometra) and peqSTAR (PEQLAB). The resulting fragments were analysed in a 1% TAE-agarose gel and purified with the GeneJET DNA Purification Kit or the GeneJET Gel Extraction Kit.

2.4.3 Mutation of conserved protein sites

Mutations were introduced into the gene sequence of the histidine kinases using primers carrying the desired mutations either in a two-step overlap PCR for cloning into a vector or in an already existing plasmid using the Q5 Site-Directed Mutagenesis Kit. Mutations were introduced in several sites of the histidine kinases: at the conserved histidine residue, in the ATP binding domain as well as the periplasmic domain. Mutations of the conserved histidine residue were based on EcoCyc *E. coli* database predictions [198]. The histidine residues were either mutated to glutamine (YedV HK) or glutamic acid (CusS).Unidentified properties like the copper sensing and the ATP binding site of CusS and YedV were inferred from sequence alignments with the related and described

histidine kinase EnvZ [71] (Figure 7.2.1 in the Appendix). The residues likely to be involved in the binding of ATP were mutated to alanine.
CusS signaling is known to be activated by copper but specific residues responsible for the sensing of copper have not been described yet whereas they have been for the CinS histidine kinase of *Pseudomonas putida* [199]. A sequence alignment of the periplasmic sensory domain of CinS from *P. putida* and of CusS from *E. coli* was used to identify residues involved in the sensing of copper. Three histidine residues of CusS were similar to the identified residues H37 and H147 of CinS and were therefore chosen as targets for mutation. These histidine residues were mutated to arginine.

2.4.4 Introduction of mutations using overlap PCR

Four primers were used to introduce a point mutation via PCR into a gene sequence. The primers were designed as follows: two forward primers, one containing the start codon of the desired gene (a), one that is binding in the gene sequence and carries the desired mutation (c); two reverse primers, one containing the stop codon of the gene (d), the other reverse complement to the second forward primer containing the mutation (b). PCRs using the primer pairs a & b and c & d were purified to get rid of the template DNA. Purification was either done using the GeneJET DNA Purification Kit or the GeneJET Gel Extraction Kit depending on whether genomic DNA or a plasmid or PCR product was used as template for the reaction. The purified PCR fragments were merged in a PCR reaction and amplified with the gene flanking primers a & d. The PCR product was tested on an agarose gel, the band with the correct size was excised, purified and used for further cloning.

2.4.5 Introduction of mutations into plasmid DNA

Point mutations were introduced into plasmid DNA using primers carrying the desired mutations and the Q5 Site-Directed Mutagenesis Kit. A PCR amplifying the entire plasmid in one go was performed according to the manufacturer's manual. Afterwards the PCR the sample was treated with an enzyme mix containing a ligase, a kinase and *DpnI* to get rid of the template plasmid and transformed into highly competent cells provided by the manufacturer.

2.4.6 Restriction Digest

PCR products and plasmids were treated with the restriction enzymes *Eco*RI, *Hind*III, *Nco*I, *Spe*I and *Xba*I from New England Biolabs or ThermoFisher Scientific. The samples were prepared according to the manufacturer's recommendation for buffers, enzyme concentration (setup see below) and incubated for 1-3 h at 37 °C. If possible the restriction enzymes were exposed to inactivation at high temperatures between 65 °C and 80 °C or if not possible purified using the GeneJET DNA Purification Kit.

Setup of digestion	10 µL	DNA
	3 µL	10x reaction buffer
	1 µL	For each restriction enzyme (or according to the manufacturer's recommendation)
	up to 30 µL	ddH_2O

2.4.7 Phosphatase treatment

The digested plasmid DNA was additionally treated with a phosphatase from NEB at 37 °C for 30 min to remove the phosphate group of the fragment and prevent religation.

Phosphatase treatment	1 µg	cut plasmid DNA
	2 µL	10x Antarctic Phosphatase Reaction buffer
	1 µL	antarctic phosphatase
	up to 20 µL	ddH_2O

2.4.8 Ligation

Ligation of digested DNA fragments and linearized plasmid DNA was performed using a T4 DNA ligase from ThermoFisher Scientific for 1 h at room temperature and then used for transformation of chemical competent cells.

Ligation reaction	20 ng	digested vector DNA
	5x molar ratio of vector DNA	digested insert
	2 µL	10x T4 DNA ligase buffer
	0.2 µL	T4 DNA ligase
	up to 20 µL	ddH_2O

2.5 Competent cells

2.5.1 Preparation of chemical competent cells

Competent cells of the knockout strains were prepared using the magnesium chloride/ calcium chloride method. Cells were grown in variable volumes of LB up to an OD_{600} between 0.6 and 0.8. After harvesting the cells by centrifugation a volume equal to the previous volume of the culture of ice-cold 0.1 M $MgCl_2$ was used to resuspend the cell pellet. An incubation step of 20 min on ice and an additional centrifugation step was followed by addition of ½ volume of 0.1 M $CaCl_2$. Cell pellets were resuspended in 0.1 M $CaCl_2$ with 18 % glycerol and either used for transformation or were shock-frozen in liquid nitrogen and stored at -80 °C.

In this work MG1655 and DH5α wildtype strains were prepared by the lab assistant using a rubidium chloride method which is similar to the calcium chloride method described above but is using two buffers containing $CaCl_2$,$RbCl_2$, Glycerin and either potassium acetate and $MnCl_2$ or sodium MOPS buffer instead.

2.5.2 Preparation of competent cells for electroporation

Electroporation was used for transformation of linear DNA into cells for genomic integration. MG1655 pKD46 cultures were then grown in LB+Amp at 30 °C to an OD_{600}= 0.2 and induced with the addition of 10 µL Ara/ 10 mL culture. When the cultures reached an OD_{600}= 0.7 to 0.8 they were put on ice for 30 min. Cells were harvested and first an equal volume of cold 10 % glycerol was added. This step was repeated with ½ and ⅕ volume glycerol. Finally cells were resuspended in 1/ 166 of the initial volume, shock-frozen in liquid nitrogen and stored at -80 °C.

2.5.3 Transformation of chemical competent cells

50 µL of thawed chemical competent cells were mixed with 1 µL plasmid DNA and left on ice for 20 minutes. Afterwards a 45 sec long heat-shock at 42 °C was applied and the cells were put back on ice for 15 min. 1 mL LB medium was added and the cells were shaken at 800 rpm and the required incubation temperature in the thermoshaker for 1 h.

Afterwards the cells were pelleted and the supernatant was removed leaving 100 µL medium. The cells were resuspended in the leftover medium and plated on LB Agar plates with the respective required antibiotic. The plates were then incubated at the required temperature over night.

2.5.4 Transformation of electro competent cells for genomic integration

Electroporation was used to introduce the linear DNA for genomic integration into the cells. A 50 µL aliquot of thawed electro competent cells were mixed with maximum 1000 ng linear DNA. The cells were left on ice for a few minutes and then transferred to a pre-cooled cuvette. The cuvette was then wiped dry and placed into the MicroPulser^{TM} electroporator (BIO-RAD). After an electric pulse the cells were mixed with 37 °C pre-warmed LB medium. After a 1-2 h incubation while shaking the cells were spun down and plated on LB plates with kanamycin to select for the positive integrates into the genome. The plates were placed at 37 °C into the incubator over night.

2.6 Cultures

Overnight cultures were inoculated either from cryo stocks or from plates. 5 mL cultures in LB medium were grown in test tubes at 30 °C and 200 rpm. 10 mL of day cultures were inoculated 1:100 with the overnight culture and grown in 100 mL flasks at 37 °C and 200 rpm. Culture conditions used for RNA isolation, protein pull-down and promoter activity assays are described in the respective chapters.

2.7 Acceptor photobleaching experiments

To identify interacting components *in vivo* FRET (Fluorescence or Förster Resonance Energy Transfer) experiments were performed [200],[201]. This method uses a distance-dependent excitation energy transfer between a CFP and YFP fluorescence protein due an overlap of their emission and excitation spectrum. In close proximity (< 10 nm) the excitation energy from CFP is transferred to YFP and the changes in the emission of both CFP and YFP can be detected [202].

In vivo steady-state interactions were assessed by using acceptor photobleaching FRET. The FRET experiments are based on the work of our former colleague Erik Sommer who constructed many C-terminal fusions of HKs and RRs to eCFP and eYFP with a mutation of the alanine in position 206 to lysine which eliminates fluorophore dimerization [203], [204]. HK or RR CFP fusions were expressed from arabinose inducible pBAD33 vectors, YFP fusions from an IPTG inducible pTrc99a vectors in MG1655 wildtpye cells in TB Medium at 34 °C to an OD_{600} 0.45-0.5 and the expression was adjusted to below 10000 copies/ cell. The copy numbers can be found in Table 6.1.13 and Figure 7.2.4 in the Appendix. 1 mL of the culture was concentrated 20-fold, put on a 1 % phosphate buffer-agarose pad and used for microscopy where the emission of CFP was recorded. After the signal stabilized YFP was bleached with a 532 nm diode laser for 20 sec (detailed description of the microscope setup [203]). From the CFP emission before and after bleaching the FRET efficiency was calculated as follows:

$$\text{FRET efficiency} = \frac{\Delta C}{C_0}$$

ΔC being the decrease in CFP fluorescence due to energy transfer to the acceptor and C_0 being the CFP fluorescence without FRET [202]. A FRET efficiency of 0.5 % was determined to be the threshold for positive interaction by our colleague Erik Sommer [203], as a smaller value indicates that the fluorophores are farther apart than 10 nm.

2.8 Promoter activation

Promoter activation assays were performed in order to assess the activation of transcription of a plasmid borne gene promoter fused to a fast folding GFP fluorophore. Promoter-GFP fusions used are part of a library of fluorescent transcriptional reporters designed by Zaslaver and colleagues [197]. Cultures were grown in Minimal A medium at 34 °C at 200 rpm. For the stimulus screen experiments cultures were grown at 37°C in the platereader.

For analysis the OD_{600} and the GFP emission at 510 nm was measured using the Tecan 1000pro platereader and flow cytometry measurements of the cultures diluted in phosphate buffer were performed. Flow cytometers used were the BD FACScan™ and BD FACSCanto™ at the Flow Cytometry and FACS Core Facility of the ZMBH

(University of Heidelberg) as well as the BD LSRFortessa™ at the Flow Cytometry Facility of the Zentrum für Synthetische Mikrobiologie (MPI für terrestrische Mikrobiologie).

2.9 RNA Isolation and Deep Sequencing

RNA isolation was performed twice during this work. The first time the RNA isolation was performed with a phenol/ chloroform method [205] from the BW25113, Δ*cusR*$_{BW}$, Δ*baeR*$_{BW}$ and Δ*yedW*$_{BW}$ strains. 10 mL of bacterial cultures were grown in Minimal A Medium at 34 °C up to an OD_{600} 0.6 -0.7 with and without 1 mM $CuSO_4$. RNA isolation was performed by mixing the cultures with cold 9 mL ethanol and 1 mL phenol. After invertion and 2 min incubation on ice the samples were centrifuged at 4000 rpm for 15 min. The supernatant was removed and the pellets were resuspended in 1.4 mL lysis buffer and incubated in a shaker at 65 °C for 5 min. After the culture cooled to 37 °C 8 µl proteinase K per 1.6 mL lysate was added and incubated at 37 °C for one hour. After incubation the samples were centrifuged at 13000 rpm for 10 min and the supernatant was split into 800 µl aliquots. Each aliquot was treated with 800 µL phenol and incubated shaking at 65 °C. After centrifugation the upper fraction was moved to a fresh Eppendorf tube mixed with 800 µL ice-cold chloroform and inverted several times. The centrifugation step was repeated and the upper fraction was again transferred to a new tube. 1.5 mL pure ethanol mixed with 65 µL 3 M sodium acetate was added and incubated at -20 °C over night. On the next day the samples were centrifuged and after the removal of the supernatant mixed with 1 mL 80 % ethanol. The samples were pelleted and the supernatant was removed. The pellets were dissolved in fresh tubes, the RNA concentration was checked and the samples were subjected to DNAse digestion to remove DNA contamination of the RNA. For the digestion of DNA 50 µg sample was incubated for 30 min at 37 °C with 1 µL DNAse and 5 µL buffer. Afterwards 350 µL double distilled water and 500 µL of a phenol-chloroform mix were added. The samples were incubated at 65 °C for 5 minutes. The upper phase was transferred to a fresh tube after a centrifugation step and treated with 500 µL ice-cold chloroform. 1.5 mL ethanol and 65 µL sodium acetate were added after a centrifugation step and incubated at -20 °C over night. The samples were then treated with 80 % ethanol, followed by the procedure which was described above. The rRNA depletion

was performed by David Ibberson (DeepSeqLab, BioQuant, Heidelberg). The RNA sequencing was done by the sequencing facility of the GeneCore facility at the European Molecular Biology Laboratory (EMBL), Heidelberg.
The second time RNA was isolated with the EUR_X GeneMATRIX Universal RNA Purification kit. Overnight cultures were grown in 5 mL Minimal A medium at 30 °C at 200 rpm. 10 mL day cultures were inoculated 1: 100 and grown at 37°C to early exponential phase and then the expression of the plasmid-encoded components was induced by adding 10 µmol IPTG for 1 hour. The isolation was performed according to the supplier's manual and after a treatment with the TURBO DNA-free kit tested for degradation on a formaldehyde gel. The samples were depleted for rRNA and sequenced at the Max Planck-Genome centre, Cologne.

2.10 Membrane protein pull-down

To detect protein-protein interaction in the cytoplasmic membrane a pull-down with tagged histidine kinases was performed. The histidine kinase fusions YedV-CFP, CusS-YFP, EvgS-YFP, PhoR-YFP, BaeS-CFP and as negative control the chemotaxis receptor Tsr-YFP were expressed in MG1655. Cultures were grown in Minimal A medium and induced with arabinose or IPTG respectively. The tagged histidine kinase copy number was set to approximately 4000/ cell. If the copy number of the YFP tagged protein from the uninduced plasmid exceeded 4000 copies/cells CFP-fusions were used.
The amount of inducer needed to reach the desired copy number was determined beforehand. For CFP fusion proteins fluorescence microscopy and a comparison with the HCB33 pKD2 strain was used to determine the required induction level. Quantification of YFP fusions was performed using flow cytometry like described in [206]. When the cultures grew up to OD_{600}= 0.6 the cells were harvested and washed with an equal volume of TN buffer. Afterwards a mixture of TN buffer, DNase I, lysozyme and protease inhibitors was added. The samples were subjected to sonication and 18 h incubation with 0.2 % Nonidet P-40 at 4 °C to solubilize the membrane proteins. Unsolubilized material was discarded by centrifugation at 14000 g for 15 min and the supernatant was mixed with buffer-equilibrated GFP-$Trap^R$_A beads (Chromotek), which bind to YFP or CFP, and incubated at 4 °C for 1 h. After 3 washing

steps with TN buffer the beads were resuspended in TN buffer diluted with SDS-sample buffer and boiled at 98 °C for 20 min to dissociate the tagged proteins and potential interaction partners from the beads. The samples were run on a 12 % SDS gel and send for mass spectrometry analysis at the Core Facility for Mass Spectrometry and Proteomics (CFMP) at the Zentrum für Molekulare Biologie (ZMBH), Heidelberg, to identify interaction partners of the HKs. To do so short peptides with a unique amino acid sequence identified by mass spectrometry are matched to the sequence of known proteins and counted. The probability for all samples was over 95 %.

2.11 Data Analysis

2.11.1 Promoter activation and FRET data

For the promoter activation experiments- if not explicitly stated- at least three independent biological replicates were performed. FRET measurements were repeated on two to four days and on each day for most samples the FRET measurement was performed twice.

The arithmetic mean $\bar{x}$ was calculated from the values of the replicates with n being the number of replicates:

$$\overline{x} = \frac{1}{n}\sum_{i=1}^{n}\frac{x_1 + x_2 + \cdots + x_n}{n}$$

To determine the deviation between the replicate values first the standard deviation s was calculated. The standard deviation was then used to calculated the standard error $s_{\bar{x}}$:

$$s = \sqrt{\frac{\sum(x - \bar{x})}{n - 1}}$$

$$s_{\overline{x}} = \frac{s}{\sqrt{n}}$$

2.11.2 RNA sequencing data

The data analysis of the RNA sequencing projects (Chapter 3.3) was performed in the ArrayStar program using student's t-test for statistical analysis and the Benjamini

Hochberg procedure for multiple testing correction. For both RNA sequencing projects genes with a linear expression level < 1 in either of the samples were excluded from the data sets to perform quantile normalization. Genes with a $\log_2$ expression level < 1 have a low number of unique mapped reads and were excluded from the analysis as well. The data are quantile normalized and depicted with their $\log_2$ expression levels. For the analysis of the MG1655 RNA sequencing project the mean expression value of the duplicates was calculated.

As a means to study variations in the expression levels of genes between different experiments we use the fold change between one experiment serving as a control and a second experiment. Fold changes are often depicted in tables or scatter plots, where a fold change of 0 represents no changes in the gene expression between the two experiments and appears on the identity line in the middle of the plot. A fold change more than 2 can be found outside the fold lines parallel to the identity line of the scatter plot. Genes upregulated in comparison with the control experiment have a positive value in tables and can be found in the experiment's half of the scatter plot. Consequently genes on the other half of the plot are downregulated in comparison with the control experiment and have a negative value in tables.

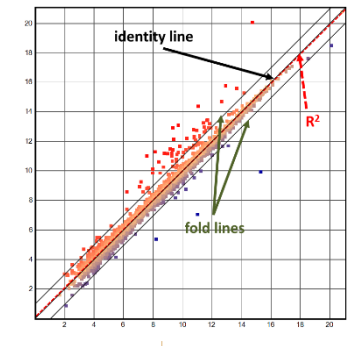

Figure 2.11.1 Scatter plot example

2.12 Software

ArrayStar	DNASTAR, Inc., USA
KaleidaGraph (version 4.03)	Synergy Software, USA
Microsoft Office, Windows 10	Microsoft, USA
SerialCloner (version 2.6.1)	Serial Basics
Scaffold (version 4.3.2)	Proteome Software Inc., USA
ImageJ	Broken Symmetry Software
Mendeley (version 1.15.3)	Mendeley, Ltd., UK

3 Results

3.1 *In vivo* interactions among TCSs

Interaction of non-cognate HKs or RRs can be an indication for cross-talk among different TCS. In order to identify cross-talk in *E. coli* TCSs I performed *in vivo* interaction experiments using acceptor photobleaching FRET. I continued and extended the work of our former colleague Erik Sommer who constructed an almost complete library of plasmids encoding for *E. coli* HKs and RRs fused to eCFP and eYFP [203]. These plasmids can be utilized in acceptor photobleaching FRET experiments and identify steady-state interactions for many cognate TCS proteins. As the number of identified interactions among non-cognate components is relatively low, acceptor photobleaching FRET can detect specific cross-interaction among the TCSs.

3.1.1 FRET measurements of interaction of cognate TCS components

To demonstrate interactions among cognate TCS components interactions of HKs with their cognate RRs and homodimerization of HKs were studied. Among cognate HK-RR pairs of several TCSs interaction had been assessed by Erik Sommer in HK-CFP/ RR-YFP and HK-YFP/ RR-CFP combinations. With a FRET efficiency threshold of 0.5 % for positive protein interaction he showed 8 out of 10 tested TCSs to exhibit interactions between their cognate components (Table 3.1.1) [203].

Table 3.1.1 FRET interaction screen among E. coli TCS components. +: FRET efficiency ≥ 0.5 %; -: FRET efficiency below 0.5 %; x: not determined. Data by Erik Sommer [203]

HK	RR	HK-YFP/ RR-CFP	HK-CFP/RR-YFP
BaeS	BaeR	+	x
BasS	BasR	+	+
CpxA	CpxR	X	+
CreC	CreB	X	-
CusS	CusR	X	+
EnvZ	OmpR	-	+
PhoQ	PhoP	+	x
QseC	QseB	+	+
RstB	RstA	X	-
YedV	YedW	+	+

The cognate HKs and the RRs of the TCSs BaeS/ BaeR, BasS/ BasR, CpxA/ CpxR, CusS/ CusR, EnvZ/ OmpR, PhoQ/ PhoP, QseC/QseB and YedV/ YedW interact in either one or in both CFP/ YFP fusion combinations. This study looked for further steady-state interactions among cognate HK-CFP/ RR-YFP pairs (Table 3.1.2).

Table 3.1.2 Steady-state interaction of cognate HK and RR pairs.
-: FRET efficiency below 0.5 % threshold.

HK-CFP/RR-YFP	FRET efficiency [%]
ArcB/ ArcA	-
BaeS/ BaeR	-
BarA/ UvrY	1.0
CitA/ CitB	1.7
CusS/ CusR	2.5
DcuS/ DcuR	2.1
EvgS/ EvgA	-
NarQ/ NarP	-
NarX/ NarL	0.7
PhoR/ PhoB	-
TorS/ TorR	1.4
UhpB/ UhpA	1.2
YedV/ YedW	4.2
YdpA/ YdpB	-

Steady-state interactions of the cognate HK and RR can be observed for more than half of the tested pairs. Among these pairs CusS/ CusR and YedV/ YedW have been shown to interact before and this finding is validated in this experiment. In summary a total of 14 out of 22 tested cognate HK-RR pairs, namely BaeS/ BaeR, BasS/ BasR, CpxA/ CpxR, CusS/ CusR, EnvZ/ OmpR, PhoQ/ PhoP, QseC/ QseB, YedV/ YedW, BarA/ UvrY, CitA/ CitB, DcuS/ DcuR, UhpB/ UhpA, TorS/ TorR and NarX/ NarL, show positive steady-state interactions.

The activation of HK signaling requires ATP hydrolysis and a dimerization of the DHp domains of two HK monomers. A screen for homo-oligomerization of cognate HKs can identify stimulus-independent interaction and was performed by Erik Sommer (Table 3.1.3) [203].

Table 3.1.3 Steady-state interaction screen among cognate *E. coli* TCS HKs.
+: FRET efficiency ≥ 0.5 %; -: FRET efficiency < 0.5 %; x: not determined. Data by Erik Sommer [203]

HK	Interaction
AtoS	+
BaeS	+
BarA	+
CitA	+
CpxA	+
CreC	-
CusS	-
DcuS	+
EnvZ	-
HydH	-
NarQ	+
PhoR	-
QseC	+
TorS	+
UhpB	+
YedV	+
YfhK	-

Homo-oligomerization can be seen for 65 % of the tested HKs. Out of 17 tested HK AtoS, BaeS, BarA, CitA, CpxA, DcuS, NarQ, QseC, TorS, UhpB and YedV self-interact even in the absence of their described stimulus. Other HKs might require the presence of their corresponding stimulus to trigger homo-oligomerization. It is also possible that the distance between the fluorophores is too wide to detect changes in FRET experiments.

3.1.2 FRET measurements of TCS cross-talk

In order to identify interactions and possible cross-talk between non-cognate HKs and non-cognate HK-RRs pairs several acceptor photobleaching FRET experiments for testing the interaction of HK-HK and HK-RR pairs were performed by Erik Sommer [203].

Hetero-oligomerization between the non-cognate HKs was studied for the HKs BaeS, DcuS, QseC, CitA, CpxA, YedV, NarQ, PhoQ and BarA. Positive interactions were found for the CpxA/ PhoQ, BaeS/ DcuS, BaeS/ YedV, BarA/ DcuS, BarA/ QseC, CitA/ CpxA, DcuS/ NarQ and DcuS/ PhoQ HK pairs, representing 13 % of the screened interaction pairs [203]. The data are comprised in Table 3.1.4 below.

Table 3.1.4 Positive steady-state interaction among non-cognate *E. coli* TCSs. Data by Erik Sommer [203].

HK-CFP	HK-YFP
BarA	DcuS
	QseC
	NarQ
DcuS	BaeS
	NarQ
	PhoQ
BaeS	DcuS
YedV	BaeS
CpxA	PhoQ
	CitA

HK-CFP	RR-YFP
UhpB	CitB
UhpB	KdpE
AtoS	CusR
BasS	QseB
CusS	YedW

HK-YFP	RR-CFP
YedV	CusR

When looking for interactions between non-cognate HKs and RRs our colleague identified 6 out of 38 tested pairs to show positive interaction [203]. The TCSs CusS/ CusR and YedV/ YedW show a double cross-interaction as the CusS and YedV HKs both interact with the RR of the respective other system.

3.1.3 Cross-talk among the CusS/ CusR, YedV/ YedW and BaeS/ BaeR TCSs

Personal communication with our collaborator Ady Vaknin (Racah Institute of Physics, Hebrew University of Jerusalem) revealed that the HKs CusS, YedV and BaeS show positive FRET homo-interaction upon stimulation with 30 µM copper. As previous findings pointed in the direction of specific cross-talk among the systems I proceeded screening for interactions among the CusS/ CusR, YedV/ YedW and BaeS/ BaeR TCSs. The CusS/CusR TCS is involved in the copper homeostasis [29] and the function of its closely related TCS YedV/ YedW is implicated [53] but not fully resolved yet. The BaeS/ BaeR TCS on the other hand is involved in envelope stress signalling and the expression of porin genes [16], [17], [20], [207].

3.1.3.1 Cognate interactions of the CusR, YedV and BaeR RRs

The TCS CusS/ CusR, YedV/ YedW and BaeS/ BaeR show homo-oligomerisation of the HKs YedV and BaeS and interaction of cognate HK-RR pairs in all three cases. The

description of the cognate component interactions was completed by performing steady-state interaction experiments for the RRs CusR, YedW and BaeR (Figure 3.1.1).

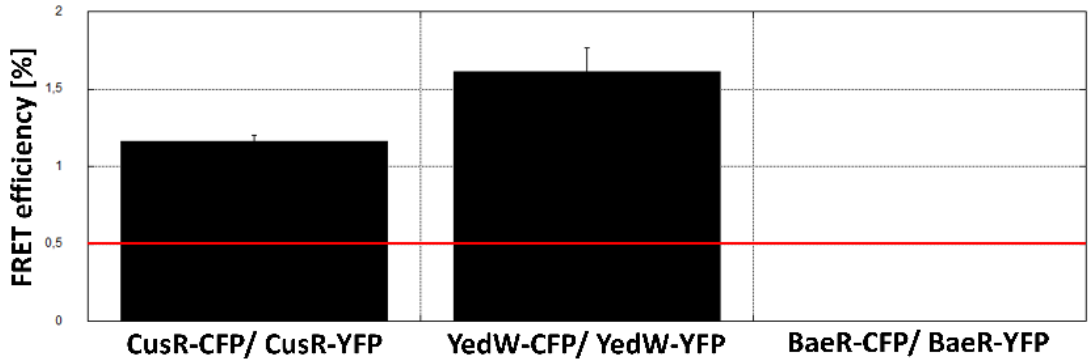

Figure 3.1.1 Steady-state homo-oligomerisation of CusR, YedW and BaeR RRs. The red line represents the 0.5 % threshold for positive interaction.

Homo-oligomerisation was identified for the CusR and YedW RRs with FRET efficiency values of 1.2 % and 1.6 %. For BaeR no change in CFP emission was detected after the photobleaching. This could be due to steric hindrance caused by the CFP/ YFP fusions to the relatively small RR protein (27.7 kDa) or because the RR monomers do not interact when they are not activated by the phosphoryl-group transfer from a HK.

3.1.3.2 Non-cognate interactions of CusS/ CusR, YedV/ YedW and BaeS/ BaeR

To study cross-interactions of the CusS/ CusR, YedV/ YedW and the BaeS/ BaeR TCSs in more detail hetero-oligomerisation on HK-HK, HK-RR and RR-RR levels were tested. I first cross-checked the interactions of the non-cognate HKs CusS, YedV and BaeS (Figure 3.1.2).

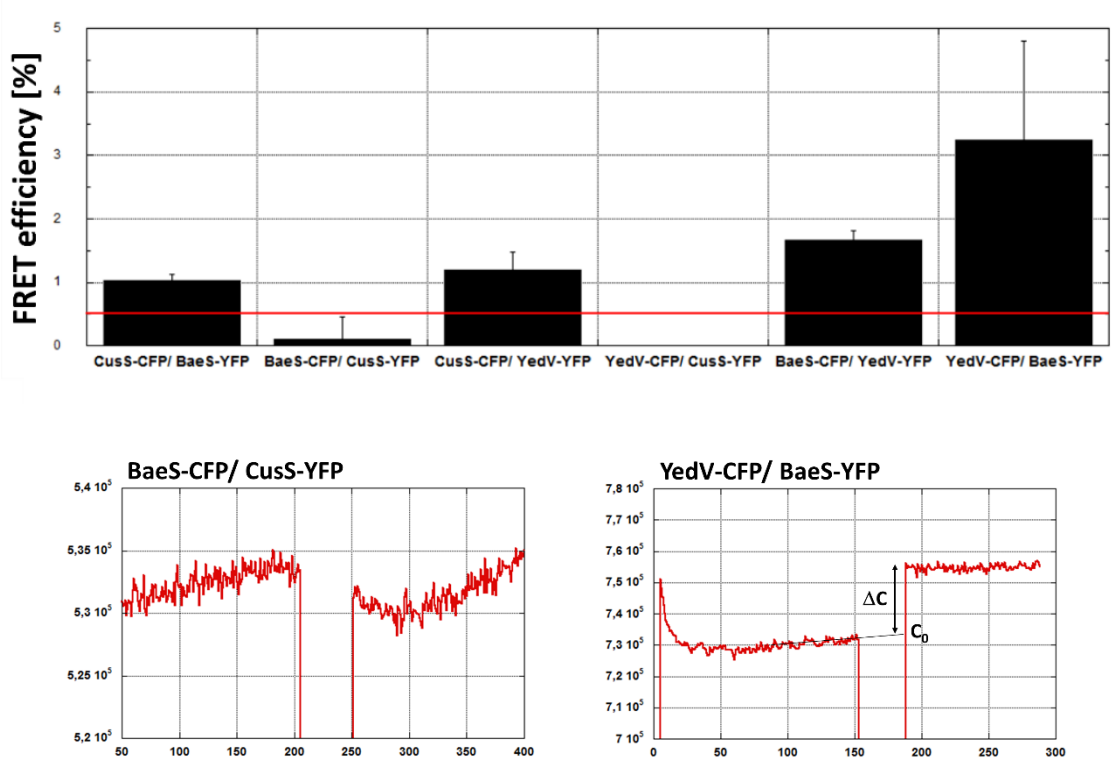

Figure 3.1.2 Steady state interaction of CusS, YedV and BaeS HKs. The red line represents the 0.5 % threshold for positive interaction. Examples for CFP fluorescence changes after YFP bleaching; BaeS-CFP/ CusS-YFP: negative interaction, YedV-CFP/ BaeS-YFP: positive interaction

The interactions of the three HK were tested in both CFP/ YFP orientations and I identified positive interaction for the CusS/ YedV, CusS/ BaeS HK pairs and confirmed the previously described interaction of YedV with BaeS. All three HKs interact with each other, strengthening the possibility of cross-talk among the TCSs.

To uncover possible cross-talk between non-cognate HKs and RRs the interactions of the HKs CusS, YedV and BaeS with non-cognate RRs of the respective other two TCSs were studied (Figure 3.1.3).

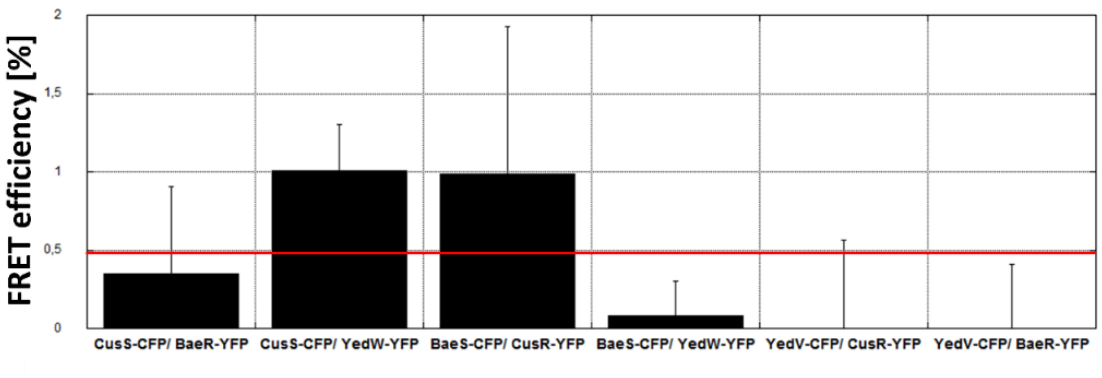

Figure 3.1.3 Steady state interaction of non-cognate HK and RRs of the CusS/ CusR, YedV/ YedW and BaeS/ BaeR TCSs. The red line represents the 0.5 % threshold for positive interaction.

The FRET experiments exhibit a positive interactions for the HK-RR pairs CusS-CFP/ YedW-YFP and BaeS-CFP/ CusR-YFP. The CusS-CFP/ BaeR-YFP HK-RR pair stayed below the threshold but could still show interaction as the standard error of the mean value is relatively high. The interactions could possibly result in a phosphoryl-group transfer among the CusS/ CusR and YedV/ YedW systems as well as among the BaeS/BaeR and CusS/ CusR systems.

I also tested for non-cognate RR interactions among CusR and YedW. Though the induction levels of the fusions have been tested previous to the microscopy experiments using flow cytometry, the CusR-CFP fusion was not sufficiently high expressed. Therefore data was acquired with the YedW-CFP/ CusR-YFP pair. The one-time experiment did not show interactions between the non-cognate RRs and therefore there is no indication for hetero-oligomerisation of CusR and YedW.

3.1.4 Pull-down of membrane-integral HKs

To identify and validate interactions between membrane-bound HKs or with other proteins which might modulate their function I performed a pull-down of the tagged HKs CusS, YedV, BaeS, EvgS and PhoR. The chemotaxis receptor Tsr and an empty MG1655 strain were used as controls. The specific isolation from the membrane fraction with antibody-beads can pull down interacting proteins which are identified by

mass spectrometry analysis. The results of the mass spectrometry analysis are shown in Table 3.1.5.

Table 3.1.5 Number of unique peptides identified by mass spectrometry analysis of HK pull-down. Copy number of probe HK set to ~ 4000 copies/ cell

Identified protein	CusS-YFP	YedV-CFP	Tsr-YFP	BaeS-YFP	EvgS-YFP	PhoR-YFP
CusS	19					
YedV		10				
Tsr			26			
BaeS				7		
EvgS					11	
PhoR						10
TnaA	20	14	2	17	4	17
Tuf2	11	5	4	6	1	5
GlpK	6	6	1	4		2
SdhA	6	4	2	4		2
AtpD	5			1		
PurA	4	1		2		1
GatZ	4	2		3	2	2
GapA	4	1	1	2		1
FliC	3	1		2		2
PutA	2					
AceA	2	2		1		3
AldA	2			3		
LacI	2					1
Lpp	2	2	2	2		
MetK	2			1		
GlyA	2			1		
SdhB	2					
AcnB	1	1		3		1
AsnS	1	1	1	4		1
Fur	1	2		2		
DnaJ			2	1		

Identified protein	CusS-YFP	YedV-CFP	Tsr-YFP	BaeS-YFP	EvgS-YFP	PhoR-YFP
OmpA			1	4	1	1
RpsQ	2	1	1	2		1
RpsC	1		1	1	1	3
RpsD	1			2		2
RpsE	2	1		1	1	
RplK	1	1		2		
RplQ	1	1	1	2	1	
RplB	4	1		3		2
RplF	1	4		7	2	3

The data set of the MG1655 pull-down serves as a positive control for the specificity of the GFP beads and therefore no positive hits were expected. Indeed it only scored hits for the highly abundant ribosomal protein RplF and therefore was excluded from the table. Pull-downs were performed for the HKs CusS-YFP, YedV-CFP, BaeS-YFP, EvgS-YFP and PhoR-YFP fusion. The additional HKs EvgS and PhoR are involved in the acid resistance [34], [208] and the response to phosphate limitation [44]–[46].

For all HKs several Rps and Rpl proteins were identified. They are part of the 30s and 50s ribosomal subunits and highly abundant in bacterial cells. Proteins interacting with CusS-YFP with more than two unique amino acid sequences identified are the flagellin subunit FliC, the adenylosuccinate synthetase PurA, the tagatose-1,6-bisphosphate aldolase GatZ, the glyceraldehyde 3-phosphate dehydrogenase GapA, the ATP synthase subunit AtpD, the glycerol kinase GlpK, the succinate:quinone oxidoreductase SdhA, the translation elongation factor Tuf2 as well as the tryptophanase TnaA (protein annotations taken from [198]). Several of the identified proteins were also pulled down with YedV-CFP (SdhA, Tuf2, GlpK and TnaA). The sample of BaeS-YFP identified aconitate hydratase 2 and 2-methylisocitrate dehydratase AcnB, GatZ, the aldehyde dehydrogenase A AldA, the asparaginyl-tRNA synthetase AsnS, GlpK, the outer membrane porin OmpA which occurs uniquely in this sample, SdhA, Tuf2 and TnaA (protein annotations taken from [198]). The pulldown of EvgS-YFP only identified TnaA with more than two unique amino acid sequences and PhoR-YFP showed AceA (isocitrate lyase), Tuf2 and TnaA (protein annotations taken from [198]). In general the

performed pull-down experiments showed only a few proteins which are associated with the probing HK. Most likely the pulled down proteins are not interacting specifically with the respective HKs. The absence of specific protein interaction could be due to a relatively low number of copies of the probes. Another factor could be a low abundance of the actual interacting proteins which therefore might not be identified in this experiment. This might especially hold true for other TCSs which could require their activating stimulus for an increase in protein copy numbers through auto-amplification.

3.2 Promoter activation experiments

RRs serve as transcription factors and gene expression can be used to access how the signaling of TCSs is influenced. To test whether component interactions have an impact on TCS signaling, promoter-GFP reporters [197] of target genes were expressed in the MG1655 wildtype and its derived knockout strains.

3.2.1 Promoter activation by CusS/ CusR and YedV/ YedW TCSs

Prior acceptor photobleaching FRET experiments (Chapter 3.1.) indicated a potential cross-talk between the CusS/ CusR and the YedV/ YedW TCSs. Therefore I investigated promoter reporter expression of the *cusC* gene (pAM96), which encodes for a subunit of a copper exporter and has been described to be controlled by the CusS/ CusR TCS [29]. I also looked at the expression of the *yedW* RR gene (pES174) and the *yedX* (*hiuH*) gene (pMF48), which has been recently described as a target gene of both YedV/ YedW and CusS/ CusR dependent on the activating stimulus [53]. For the CusS/ CusR TCS a described stimulus is copper [28], [29]. Therefore promoter-GFP reporter activation upon stimulation with 1 mM $CuSO_4$ was tested. The GFP-expression was quantified using flow cytometry and fluorescence values are stated in RFUs (random fluorescence units). Figure 3.2.1 shows the expression of the promoter-GFP reporters in MG1655 without and with a copper stimulus.

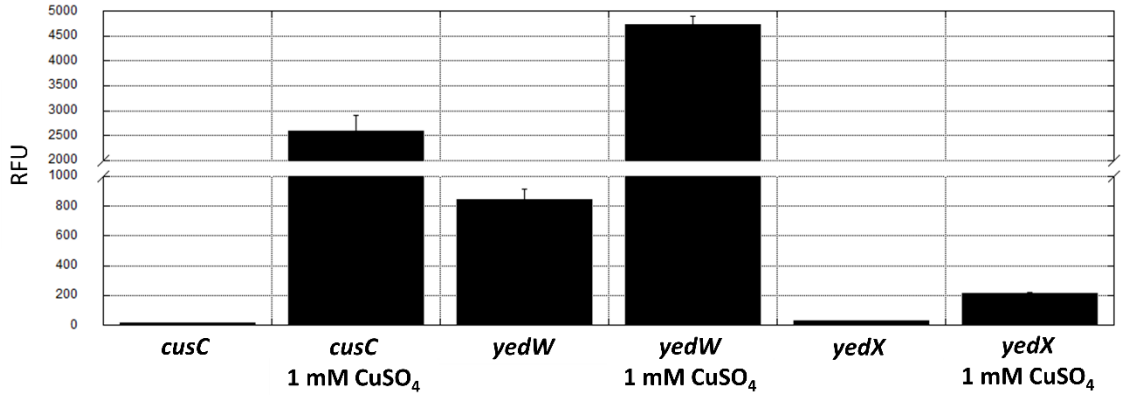

Figure 3.2.1 Activation of *cusC*, *yedW* and *yedX* (*hiuH*) promoter-GFP fusions in MG1655.

The *cusC* promoter-GFP reporter exhibits low fluorescence of 15 RFUs when grown in Minimal A medium under non-stimulated conditions. The addition of 1 mM $CuSO_4$ to the culture induces the promoter 196 times. For the promoter-GFP reporter *yedW* a high basal expression of 793 RFUs is observed, addition of 1 mM $CuSO_4$ to the culture leads to a 6 x upregulation. The *yedX* (*hiuH*) promoter's basal expression is low (27 RFUs). Addition of the copper stimulus induces its expression 8 times.

I studied which effect the lack of either component of the CusS/ CusR and YedV/ YedW TCSs or the lack of the entire CusS/ CusR TCS has on the *cusC* and *yedW* promoter reporter expression (Figure 3.2.2).

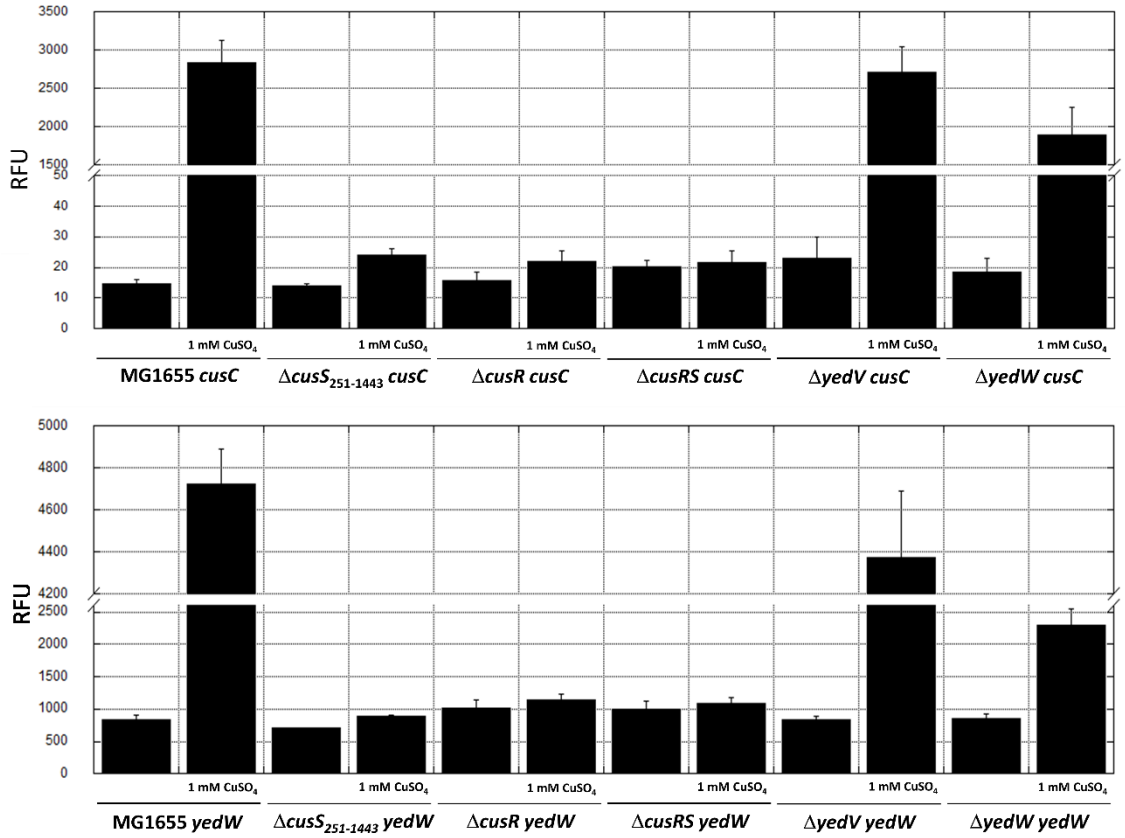

Figure 3.2.2 *cusC* and *yedW* promoter activation in MG1655, CusS/ CusR and YedV/ YedW TCS knockout strains.

The expression of the *cusC* and *yedW* promoter reporter without and with induction by a $CuSO_4$ stimulus was studied in the MG1655 wildtype and in strains lacking the CusS HK (Δ*cusS*$_{251\text{-}1443}$), the CusR RR (Δ*cusR*) and the entire TCS (Δ*cusRS*) as well as in knockout strains of the YedV/ YedW TCS genes (Δ*yedV* and Δ*yedW*). The *cusC* promoter activation is almost completely abrogated in uninduced Δ*cusS*$_{251\text{-}1443}$, Δ*cusR* and Δ*cusRS* strains with the same expression level as the uninduced wildtype. Also no changes in the expression level for Δ*cusRS cusC* + 1 mM $CuSO_4$ can be observed. Upon copper stimulation Δ*cusS*$_{251\text{-}1443}$ and Δ*cusR* show a 2 x increase in promoter expression. As the promoter expression in the wildtype increases by 196 x upon copper stimulation a 2 x increase is not very substantial. The Δ*yedV* and Δ*yedW* strains show an increase of

cusC expression of 187 x and 130 x upon stimulation with copper, which is lower than in the wildtype.
A similar pattern can be seen for the activation of the *yedW* promoter reporter, which is induced 6 times upon copper stimulation in the wildtype. No expression changes in the Δ*cusS*$_{251\text{-}1443}$, Δ*cusR* and Δ*cusRS* strains are found. A diminished activation can be observed in Δ*yedV* and Δ*yedW*, where copper stimulation leads to a *yedW* promoter reporter increase of only 5 x and 3 x.
A summarizing conclusion is that the presence of either component of the CusS/ CusR TCS is required for a sufficient activation of the *cusC* and *yedW* gene expression. The YedV/ YedW TCS seems to contribute to the overall activation of the gene expression though the absence of its components does not abrogate the gene activation.

3.2.2 Complementation of CusS deletion

A test whether the lack of *cusC* induction in Δ*cusS*$_{251\text{-}1443}$ can be restored by expressing CusS from a plasmid (pMF15) was performed. I further investigated whether the activation of CusS signaling is entirely dependent on the detection of copper by complementation with CusS HKs mutated for potential copper binding sites (pMF 38) (Figure 3.2.3).

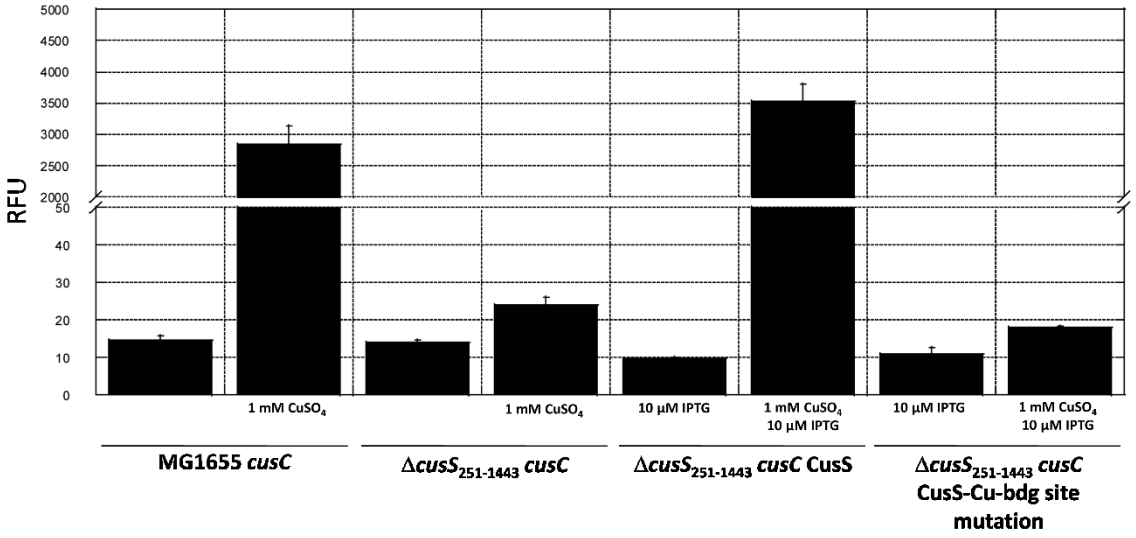

Figure 3.2.3 Complementation of cusC promoter activation in ΔcusS251-1443 by CusS HK and with CusS with modified copper binding domain. Complementations are induced with 10 µM IPTG.

Expression of CusS from a plasmid in Δ*cusS*$_{251\text{-}1443}$ restores the *cusC* promoter induction by copper to an even higher level than in the wildtype (244 x). Complementation with a CusS HKs mutated for potential copper binding sites in its periplasmic domain does not restore the copper activation of the promoter. Thus detection of copper by the periplasmic domain of CusS is essential for the activation of the signaling.

3.2.3 Effect of YedV on gene expression

TCSs often regulate their own component's transcription via auto-amplification. Usually especially the HKs, which sense the activating stimulus, show low abundance under non-stimulated conditions. An activation of the TCSs signaling therefore results in a higher HK copy number. To study how an increased number and thereby a mimicked activation of the YedV HK impacts the promoter activation I looked at the *cusC* promoter in the wildtype with expressing YedV (pMF18), YedV$_{H245Q}$ mutated for its conserved His residue (pMF23) and YedV-HATPase mutation (pMF30). The HK

CpxA (pMF29) was included as a control and all components are expressed from a plasmid (Figure 3.2.4).

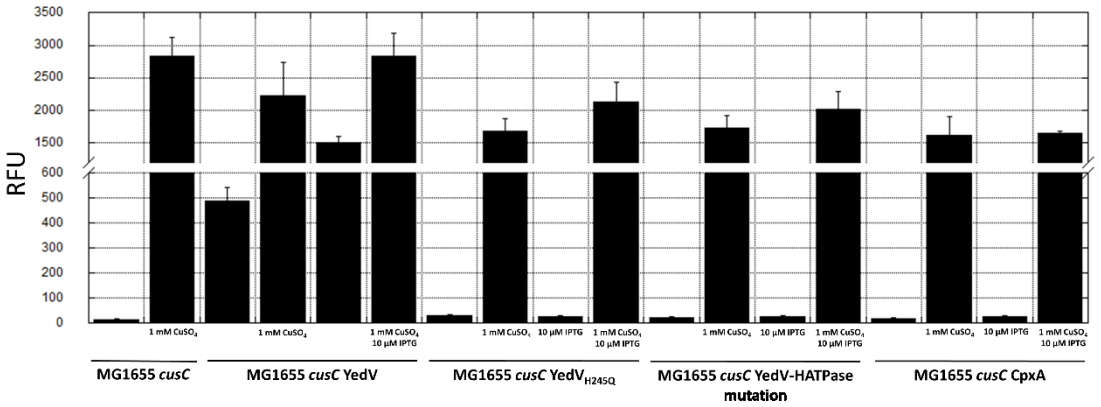

Figure 3.2.4 Effect of YedV HK and YedV mutations overexpression on *cusC* promoter activation in MG1655 wildtype; induced with 10 µM and stimulated with $CuSO_4$.

In the wildtype MG1655 YedV expression induces the *cusC* promoter reporter without copper stimulus. YedV expression from the leaky pTrc99a promoter results in a 34 x upregulation of the promoter and is further amplified upon induction with 10 µM IPTG to a change of 103 times. This effect does not occur when $YedV_{H245Q}$ (conserved His residue mutation) is expressed. The YedV with a mutation of the HATPase domain is mutated to prevent binding of and thereby hindering the initial hydrolysis of ATP which is important for the phosphorylation of the HK. YedV-HATPase mutation and the HK CpxA show a similar pattern as $YedV_{H245Q}$. Here an upregulation upon stimulation with $CuSO_4$ is observed but non to only 2 x when the culture is induced with 10 µM IPTG. The excess of the YedV HK is sufficient to induce upregulation of the CusS/ CusR target gene *cusC*. But only a fully functional YedV HK can induce the *cusC* promoter reporter expression as the lack of either of the conserved His residue ($YedV_{H245Q}$) or the HATPase domain fails to generate the same effect. The activation of HK signaling requires both the HATPase domain and the conserved His residue and the mutations should render the HK non-functional. The activation of *cusC* expression is also specifically induced by YedV as the excess of the HK CpxA does not result in a copper-independent induction of the *cusC* promoter reporter. Strains expressing the YedV mutations show a lower promoter activation than the wildtype. A lack of the YedV HK

yields a lower promoter activation in the Δ*yedV* strain. In this experiment the YedV mutations are expressed in the MG1655 wildtype which also encodes a functional YedV HK and therefore should show wildtype like promoter activation. However the lower activation could result from interactions of the low number of functional YedV with the highly abundant non-functional YedV. This could lead to the formation of non-functional HK dimers and thereby creating an almost Δ*yedV*-like effect on the promoter activation. The excess CpxA HK on the other hand could indirectly lower the *cusC* promoter activation by draining ATP from the CusS HK which is required for its autophosphorylation step.

An alternative possibility is that the YedV mutations instead of being non-functional could also retain a certain level of HK activity. Thereby YedV mutation expression could lead to a lower level of non-specifically phosphorylated CusR which can be counteracted by phosphatase activity of the CusS HK. Whereas a fully functional YedV HK could increase CusR phosphorylation to a level which cannot be compensated for by CusS. Upon stimulation with copper the dimerization of functional YedV with mutated YedV could explain the lower *cusC* promoter reporter expression.

To pinpoint whether the YedV HK expression influences the *cusC* promoter activation on the level of the CusS HK, the level of the CusR RR or by YedW induced target gene transcription YedV and its mutations were expressed in the Δ*cusS*$_{251\text{-}1443}$ strain (Figure 3.2.5).

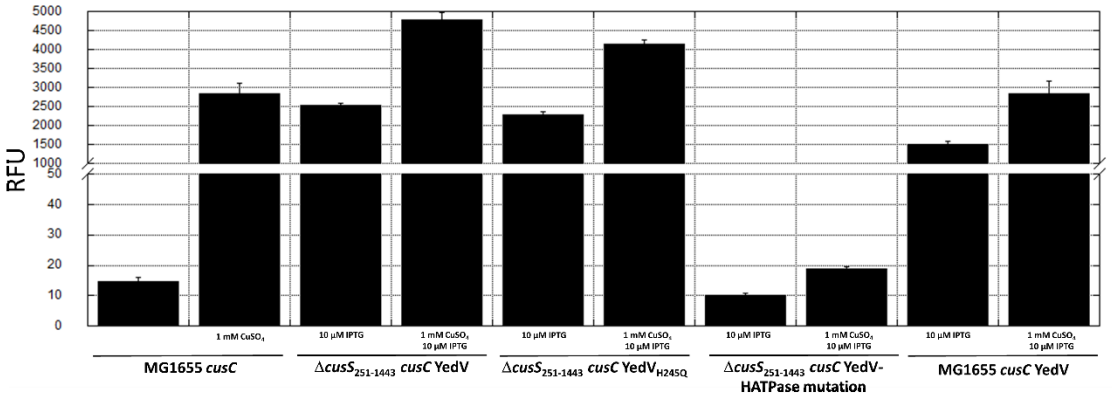

Figure 3.2.5 *cusC* promoter activation in MG1655 and Δ*cusS*$_{251\text{-}1443}$ by YedV HK and with YedV with mutated conserved histidine residue or YedV HATPase domain. Expression of HKs are induced with 10 µM IPTG

In Δ*cusS*$_{251\text{-}1443}$ the lack of the CusS HK leads to a further increase in *cusC* promoter reporter expression by YedV. The changes of the *cusC* promoter reporter expression in the MG1655 wildtype are 103 x and 196 x upon induction of the YedV expressing plasmid with 10 µM IPTG and with both 10 µM IPTG and 1 mM $CuSO_4$. In Δ*cusS*$_{251\text{-}1443}$ the expression under the same conditions is 176 x and 329 x. This indicates that normally CusS works as a phosphatase upon YedV expression and that phosphorylation of the CusS HK by YedV does not play a role in the activation step. The YedV HK with a mutation of the HATPase domain fails to activate the promoter reporter expression in Δ*cusS*$_{251\text{-}1443}$ almost entirely. The mutation of the conserved His residue in YedV$_{H245Q}$ on the other hand shows similar but slightly lower changes in expression levels (158 x and 285 x) than the fully functional YedV HK. An explanation could be the formation of dimers between functional YedV and YedV$_{H245Q}$ which still can catalyse the phosphorylation of the conserved His residue in *trans*. The subsequent phosphorylation of CusR would not be compensated for by CusS's phosphatase activity and therefore could induce the *cusC* reporter higher than in the wildtype. The almost 2 x upregulation of *cusC* in the Δ*cusS*$_{251\text{-}1443}$ with overexpressed YedV and its mutants upon copper stimulation is an interesting observation. As the data (Figure 3.2.2) does not support copper-sensing by the YedV HK this effect is most likely not due to direct recognition of copper. Also the periplasmic domains of CusS and YedV are distinctly different and do not share the copper-binding residues, lowering the probability of YedV sensing copper. Rather likely is the activation and thereby amplification the YedV HK seems to be activated by an unknown input signal which might be related to copper stress.

The YedV HK seems to independently either cross-phosphorylate the CusR RR or by linear signaling within the YedV/ YedW TCS activate *cusC* expression. As a one-time experiment with YedW expression (pMF 19) in Δ*cusS*$_{251\text{-}1443}$ showed only a slight higher *cusC* promoter reporter expression (3-fold) than in Δ*cusS*$_{251\text{-}1443}$ *cusC* the YedV signaling is more likely to happen through cross-phosphorylation of CusR.

3.2.4 Screen for YedV/ YedW activating stimulus

Several different compounds were tested for an activation of the promoter reporter *cusC*, *yedW* and *yedX* (*hiuH*) to identify stimuli which activate the YedV/ YedW TCS. Screening for perception of stimuli were performed in 24-well plates with MG1655 wildtype strain bearing the respective promoter-GFP reporter plasmid [197] or additionally express YedWV from a second plasmid (pMF34). For stimuli which formed a precipitate in Minimal A medium cultures were grown in LB medium. In the screen growth and fluorescence were measured over several hours time and analysed the results of the plate reader measurements by dividing the fluorescence by the OD_{600} of the culture. Given that $CuSO_4$ activates the CusS/ CusR TCS and the YedV/ YedW TCS was found to be involved with its signaling I tested other divalent cations like for instance silver, tin and nickel. I also investigated whether intermediate compounds of the purine metabolism [53], [190], [209] and compounds which cause oxidative stress have an effect on promoter reporter expression. The tested stimuli are Ag_2SO_4, $MgCl_2$, $CrCl_2$, $MnCl_2$, $FeSO_4$, $CoCl_2$, $NiCl_2$, $ZnCl_2$, $Pb(NO_3)_2$, H_2O_2, uric acid, TMAO (trimethylamine N-oxide) and urea. The stimuli were tested in different concentrations mostly ranging from 10 µM to 1 mM or in the case of H_2O_2 0.6 mM and higher. For the tested stimuli no obvious changes in the promoter expression of either tested promoter reporter was observed. It has to be mentioned that the gain chosen to prevent overflow in the fluorescence channel caused by cell growth could be a reason low changes in promoter activation are not detected as a higher gain results in decrease in detection sensitivity for low levels of GFP expression. Under the tested conditions I could not identify a novel stimulus which can activate the YedV/ YedW TCS signaling.

3.3 Transcriptomic analysis of TCS

To delineate target gene expression and regulation by TCSs RNA isolation and sequencing was performed. Initially RNA isolation was performed for one sample each of the BW25113 wildtype, Δ$cusR_{BW}$, Δ$yedW_{BW}$ and Δ$baeR_{BW}$ to characterize genes controlled by the CusS/ CusR, YedV/ YedW and the BaeS/ BaeR TCSs dependent or independent of stimulation with $CuSO_4$. Genes downregulated in the knockout strains Δ$cusR_{BW}$, Δ$yedW_{BW}$ and Δ$baeR_{BW}$ can potentially be positively controlled by the RRs in

the wildtype and vice versa. Later duplicates of samples with YedV/ YedW components and the CusR RR expressed from a plasmid in MG1655, Δ*cusR*$_{MG}$ and Δ*cusRS*$_{MG}$ were sequenced. As several studies have shown that for instance overexpression of RR can activate and amplify target genes expression independent from the activating stimulus and the phosphorylation state of the RR [12], [54], [210] this method is a good means to identify genes regulated by a TCS of unknown function. YedV (pMF18) and YedWV (pMF34) were expressed in all three strains, YedW (pMF19) was expressed in Δ*cusRS*$_{MG}$ and Δ*cusR*$_{MG}$ and CusR (pMF16) was expressed in the MG1655 wildtype. As a reference an empty pTrc99a plasmid (pVS198) was expressed in all three strains and are referred to as MG1655, Δ*cusR*$_{MG}$ and Δ*cusRS*$_{MG}$ in this chapter. In strains overexpressing components a positive fold change can be interpreted as an indication for positive regulation by the respective component.
A description of how the data analysis was performed can be found in Chapter 2.11.2 and scatter plots and linear correlation coefficients of the duplicates are shown in Figure 7.2.2 and in the Tables 7.1.5 and 7.1.6 in the Appendix.

3.3.1 Gene regulation by the CusS/ CusR TCS

To investigate the CusS/ CusR TCS in more detail RNA sequencing of a CusR deficient strain (Δ*cusR*$_{BW}$) and the wildtype strain BW25113 with and without its stimulus copper was performed. I also performed sequencing for a set of two samples overexpressing CusR in the MG1655 wildtype to infer genes regulated by CusR independent of a stimulus.

3.3.1.1 Copper effect on gene expression in Δ*cusR*$_{BW}$

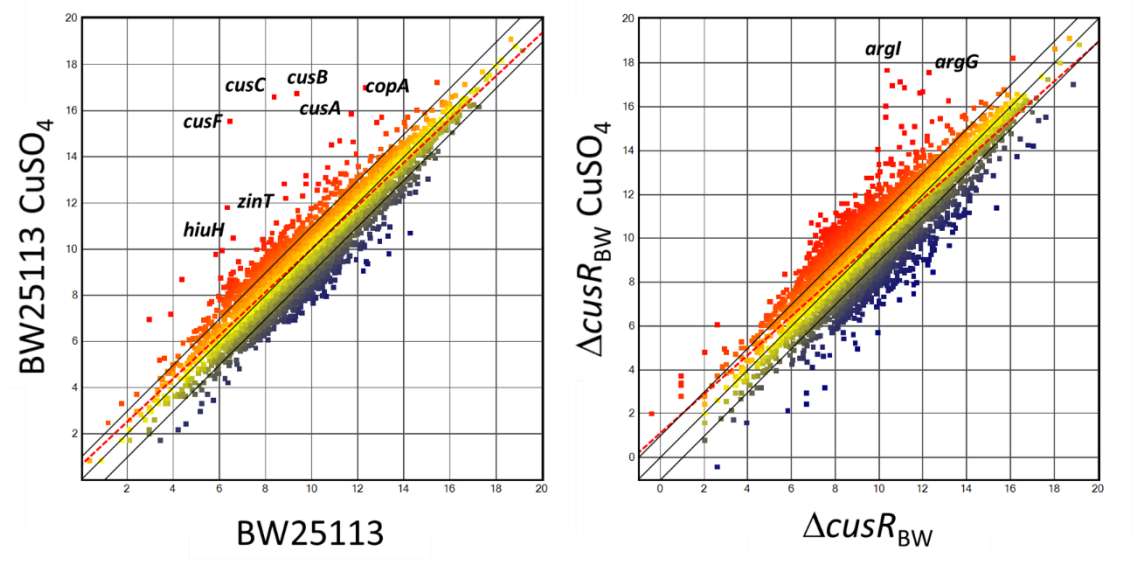

Figure 3.3.1 Scatter plots of gene expression in BW25113 and Δ*cusR*$_{BW}$ without and with $CuSO_4$ stimulus.

The scatter plots of the BW25113 wildtype and the Δ*cusR*$_{BW}$ strain in Figure 3.3.1 show the gene expression pattern upon stimulation with copper. In the wildtype several genes are clearly upregulated upon copper stimulation. Among them are the *cusC*, *cusF*, *cusB* and *cusA* genes, which are organized in one operon, as well as *copA*, *hiuH* and *zinT* which are upregulated as well. Both *cusCFBA* and *copA* encode for copper exporters [188], [184], *hiuH* encodes for a hydroxyisourate hydrolase [190], [198] and *zinT* encodes for a cadmium binding protein which binds divalent metal ions [198]. A slight upregulation of *arg* genes which are involved in the arginine biosynthesis [198] can be observed in Δ*cusR*$_{BW}$ which corresponds with a study performed in *B. subtilis* which

found that arginine biosynthesis genes are downregulated in the wildtype during copper stress but upregulated in strains deficient for a global regulator of copper homeostasis [178]. In the scatter plots of the wildtype and Δ*cusR*$_{BW}$ a slight downregulation of some genes which are not indicated in the figure can be observed. The genes *hdeAB*, *gadA* and *gadBC* which are involved in acid stress response are downregulated about 13-fold in BW25113 background. In Δ*cusR*$_{BW}$ *dps*, *ndk* and *cheY* genes are downregulated also around 14-fold. They are involved in DNA protection in response to oxidative and other stresses, transfer of phosphate to nucleotides and chemotaxis. A more detailed analysis of gene fold changes upon copper stimulation is viewed in Table 3.3.1 (fold change more than 8) and Table 7.1.11 (fold change between 4 and 8) in the Appendix.

Table 3.3.1 Fold changes more than 8-fold in BW25113 and Δ*cusR*$_{BW}$ without and with $CuSO_4$. Table sorted according to the fold changes in BW25113.

Gene	Annotation	BW25113 → BW25113 $CuSO_4$	Δ*cusR*$_{BW}$ → Δ*cusR*$_{BW}$ $CuSO_4$
cusF	periplasmic copper- and silver-binding protein	555.9	-1.0
cusC	copper/silver efflux system, outer membrane component	305.7	1.5
cusB	copper/silver efflux system, membrane fusion protein	177.6	2.4
zinT	zinc and cadmium binding protein, periplasmic	46.2	14.2
copA	copper transporter	26.7	28.4
ykgO	RpmJ-like protein	20.5	3.9
cusA	copper/silver efflux system, membrane component	18.2	6.1
trpL	trp operon leader peptide	16.8	-1.5
yebE	inner membrane protein, DUF533 family	16.5	8.8
ykgM	50S ribosomal protein L31 type B, alternative L31 utilized during zinc limitation	15.3	4.2
hiuH	hydroxyisourate hydrolase	14.9	-3.4
yncJ		14.6	3.3
znuA	zinc transporter subunit: periplasmic-binding component of ABC superfamily	13.1	5.7
cpxP	inhibitor of the cpx response, periplasmic adaptor protein	11.4	2.2
cusR	DNA-binding response regulator in two-component regulatory system with CusS	11.2	-7.9
spy	periplasmic ATP-independent protein refolding chaperone, stress-induced	10.6	7.5
cspI	Qin prophage, cold shock protein	10.2	5.6
pyrI	aspartate carbamoyltransferase, regulatory subunit	8.6	4.3
gadA	glutamate decarboxylase A, PLP-dependent	-8.2	1.8
hdeB	acid-resistance protein	-8.9	-4.3
gadB	glutamate decarboxylase B, PLP-dependent	-11.4	-1.1
hdeA	stress response protein acid-resistance protein	-12.1	-5.7

As indicated by the scatter plots in Figure 3.3.1 the *cusC*, *cusF* and *cusB* genes exhibit a high positive fold changes above 177-fold in the BW25113 wildtype upon $CuSO_4$ stimulation. This is abrogated in Δ*cusR*$_{BW}$ where no upregulation upon stimulation with copper can be observed. A similar expression profile can be found for *hiuH*. Its expression increases by 15-fold in the wildtype sample compared to the Δ*cusR*$_{BW}$ sample where it has a negative fold change of -3.4. The expression of *copA* does not change in Δ*cusR*$_{BW}$ upon copper stimulation confirming an independent function of the CueR-regulon. Genes upregulated in BW25113 which have a lower fold change in Δ*cusR*$_{BW}$ → Δ*cusR*$_{BW}$ $CuSO_4$, are likely to be regulated by CusR in a copper-dependent manner. The differences in fold change range from 5.3 x to 2.1 x lower than in the wildtype for the genes *ykgO*, *cpxP*, *ykgM*, *zinT* and *znuA*. The genes *zinT* and *znu*A are for instance involved in the cell's response to zinc. CpxP is responsible for the repression of the signaling of the CpxA/ CpxR TCS which responds to various stresses [211]. Genes with a fold change below zero could be potentially negatively regulated by CusR. The fold changes in the wildtype and Δ*cusR*$_{BW}$ vary between 10.4 x and 2.1 x. for the genes *gadA*, *gadB*, *hdeA* and *hdeB* which are involved in the resistance to acidic conditions. Most genes, especially those with a fold change less than 8 (Table 7.1.11), show only minor changes between the wildtype and the knockout strain or a partial abrogation of the increase in expression upon copper stimulation. This might not be due to the lack of the CusR RR but due to small differences in gene expression between the two cultures.
Interestingly the HK *cusS* gene's upregulation in the wildtype upon stimulation with copper is only 6.1-fold and it can be concluded that the expression and thereby the copy number of the CusS HK does not increase dramatically upon activation of the TCS. Other genes affected are for instance involved in the transport of carbohydrates, organic acids and alcohols, flagellar components, part of the chemotaxis system and ATPases.

3.3.1.2 Effect of *ΔcusRS*$_{MG}$ and *ΔcusR*$_{MG}$ on gene expression

Aside from the effect the lack of the CusR RR has on the gene expression in response to copper I investigated how the CusS/ CusR TCS influences the gene expression in a non-copper-induced manner. Two strains, one lacking the RR gene *cusR* and the other

defective for the entire TCS operon *cusRS* in a MG1655 background were used to perform RNA sequencing analysis. Gene expression patterns and fold changes between the MG1655 wildtype, Δ*cusRS*$_{MG}$ and Δ*cusR*$_{MG}$ strains are depicted in Figure 3.3.2 and Table 3.3.2. A list of genes sorted by their fold changes in Δ*cusR*$_{MG}$ can be found in Table 7.1.10 in the Appendix.

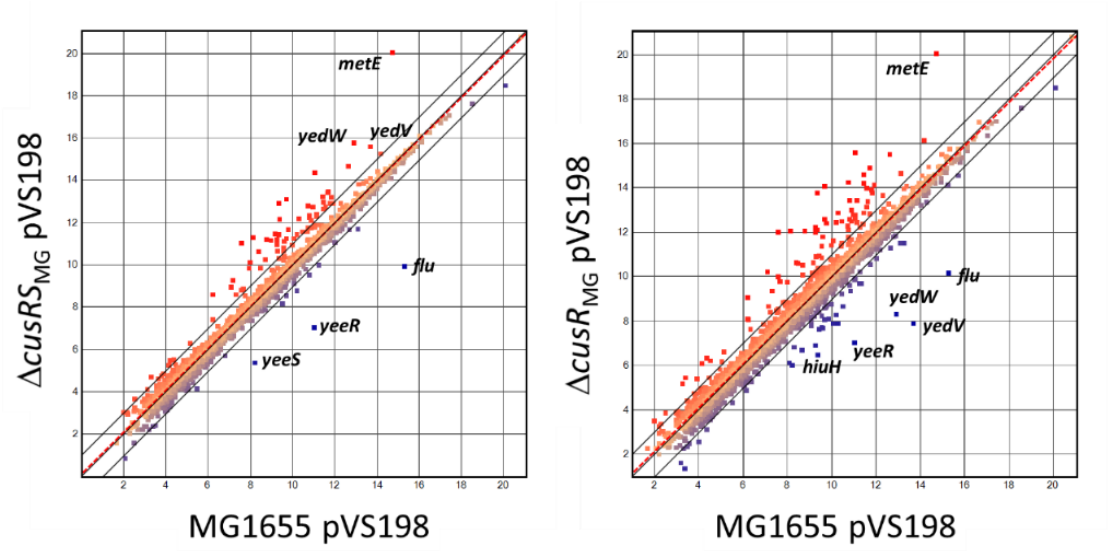

Figure 3.3.2 Scatter plots of gene expression between MG1655 pVS198 and Δ*cusRS*$_{MG}$ pVS198 or Δ*cusR*$_{MG}$ pVS198.

The scatter plots of both *ΔcusRS*$_{MG}$ and *ΔcusR*$_{MG}$ compared to the wildtype show a downregulation of the genes *yedW*, *yedV* and *hiuH* in the *ΔcusR*$_{MG}$ strain whereas *yedW* and *yedV* are slightly upregulated if the entire CusS/ CusR TCS system is defective. This might be due to a CusR-independent role of the CusS HK in the regulation of these genes. Also a downregulation of *flu*, *yeeR* and *yeeS* genes in the knockout strains can be observed which does not occur in the wildtype. The three genes are organised in one operon. The *flu* gene encodes for the highly abundant surface antigen Ag43 which is involved in the autoaggregation of cells and exhibits an expression pattern which undergoes phase variable switching [212], [213]. An upregulation of *metE* in the

knockout strains can be noted as well. MetE is a methionine synthase involved in the final step of the methionine biosynthesis. It has been shown to be inactivated by transient oxidative stress when cells are grown in minimal medium [214] and therefore the observed regulation might be due to slightly different oxygen availability in the cultures. Overall there are only a few genes affected by the deletion of the CusS/ CusR TCS when grown in minimal A medium under non-inducing conditions.

The fold changes of *ΔcusRS*$_{MG}$ and *ΔcusR*$_{MG}$ compared to the wildtype are depicted in Table 3.3.2 and Table 3.3.3. Scatter plots of MG1655 pVS198 compared to Δ*cusRS*$_{MG}$ and Δ*cusR*$_{MG}$ can be found in the Appendix Figure 7.2.3.

Table 3.3.2 Fold changes more than 4-fold between MG1655 pVS198 → Δ*cusRS*$_{MG}$ pVS198.

Gene	Annotation	MG1655 pVS198 → Δ*cusRS*$_{MG}$ pVS198	MG1655 pVS198 → Δ*cusR*$_{MG}$ pVS198
flu	CP4-44 prophage, antigen 43 (Ag43) phase-variable biofilm formation autotransporter	-39.8	-33.7
yeeR	CP4-44 prophage, predicted membrane protein	-15.2	-15.9
yeeS	CP4-44 prophage, predicted DNA repair protein	-6.9	-4.6
cysD	sulfate adenylyltransferase, subunit 2	4.2	11.6
metQ	DL-methionine transporter subunit	4.2	7.6
ybdH	putative oxidoreductase	4.4	7.0
rrsD	16S ribosomal RNA of rrnD operon	4.4	2.9
rrsA	16S ribosomal RNA of rrnA operon	4.5	3.4
yedY	membrane-anchored, periplasmic TMAO, DMSO reductase	4.7	-1.7
metR	DNA-binding transcriptional activator, homocysteine-binding	4.8	5.0
rrsC	16S ribosomal RNA of rrnC operon	5.1	2.9
ybdM		5.3	7.4
metA	homoserine O-transsuccinylase	5.6	10.1
hiuH	hydroxyisourate hydrolase	7.0	-7.4
yedW	putative DNA-binding response regulator in two-component system with YedV	7.4	-23.5
ybdL	methionine aminotransferase, PLP-dependent	9.1	15.8
metL	fused aspartokinase II/homoserine dehydrogenase II	10.1	23.1
metF	5,10-methylenetetrahydrofolate reductase	10.7	21.0
mmuP	CP4-6 prophage, predicted S-methylmethionine transporter	11.0	21.6
metB	cystathionine gamma-synthase, PLP-dependent	12.1	22.0
metE	5-methyltetrahydropteroyltriglutamate- homocysteine S-methyltransferase	41.5	41.5

Table 3.3.3 Fold changes more than 4-fold between MG1655 pVS198 → Δ*cusR*$_{MG}$ pVS198.

Gene	Annotation	MG1655 pVS198 → Δ*cusR*$_{MG}$ pVS198	MG1655 pVS198 → Δ*cusRS*$_{MG}$ pVS198
yedV	putative sensory kinase in two-component regulatory system with YedW	-53.9	3.8
flu	CP4-44 prophage, antigen 43 (Ag43) phase-variable biofilm formation autotransporter	-33.7	-39.8
yedW	putative DNA-binding response regulator in two-component system with YedV	-23.5	7.4
yeeR	CP4-44 prophage, predicted membrane protein	-15.9	-15.2
hiuH	hydroxyisourate hydrolase	-7.4	7.0
carA	carbamoyl phosphate synthetase small subunit, glutamine amidotransferase	-5.1	-1.5
xanP	xanthine permease	-5.0	-1.7
yeeS	CP4-44 prophage, predicted DNA repair protein	-4.6	-6.9
codB	cytosine transporter	-4.4	-1.6
iaaA	Isoaspartyl peptidase	4.1	2.2
iraD	RpoS stabilizer after DNA damage, anti-RssB factor	4.1	2.3
csgB	curlin nucleator protein, minor subunit in curli complex	4.1	-1.2
yiiX	putative lipid binding hydrolase, DUF830 family, function unknown	4.2	2.4
ysgA	putative hydrolase	4.4	2.3
cbl	DNA-binding transcriptional activator for the ssuEADCB and tauABCD operons	4.4	2.3
metJ	DNA-binding transcriptional repressor, S-adenosylmethionine-binding	4.4	2.6
yeeE	inner membrane protein, UPF0394 family	4.4	2.2
cysA	sulfate/thiosulfate transporter subunit	4.6	2.0
metC	cystathionine beta-lyase, PLP-dependent	4.7	3.0
cysH	3'-phosphoadenosine 5'-phosphosulfate reductase	4.8	2.4
metR	DNA-binding transcriptional activator, homocysteine-binding	5.0	4.8
cysW	sulfate/thiosulfate ABC transporter subunit	5.1	2.2
cysI	sulfite reductase, beta subunit, NAD(P)-binding, heme-binding	5.4	2.3
ygbE	DUF3561 family inner membrane protein	5.5	2.4
metI	DL-methionine transporter subunit	6.2	3.9
metN	DL-methionine transporter subunit	6.4	3.8
cysU	sulfate/thiosulfate ABC transporter permease	6.5	2.8
ybdH	putative oxidoreductase	7.0	4.4
cysJ	sulfite reductase, alpha subunit, flavoprotein	7.0	2.9
mmuM	CP4-6 prophage, S-methylmethionine:homocysteine methyltransferase	7.0	3.9
ybdM		7.4	5.3
cysC	adenosine 5'-phosphosulfate kinase	7.4	3.0
metQ	DL-methionine transporter subunit	7.6	4.2
yciW	putative oxidoreductase	8.0	3.4
sbp	sulfate transporter subunit	8.7	3.7
cysP	thiosulfate-binding protein	9.1	3.5

Gene	Annotation	MG1655 pVS198 → Δ*cusR*MG pVS198	MG1655 pVS198 → Δ*cusRS*MG pVS198
cysN	sulfate adenylyltransferase, subunit 1	9.5	3.5
metA	homoserine O-transsuccinylase	10.1	5.6
cysD	sulfate adenylyltransferase, subunit 2	11.6	4.2
ybdL	methionine aminotransferase, PLP-dependent	15.8	9.1
metF	5,10-methylenetetrahydrofolate reductase	21.0	10.7
mmuP	CP4-6 prophage, predicted S-methylmethionine transporter	21.6	11.0
metB	cystathionine gamma-synthase, PLP-dependent	22.0	12.1
metL	fused aspartokinase II/homoserine dehydrogenase II	23.1	10.1
metE	5-methyltetrahydropteroyltriglutamate- homocysteine S-methyltransferase	41.5	41.5

Most of the genes up- or downregulated more than 4-fold in MG1655 pVS198 → Δ*cusRS*MG pVS198 are also regulated more than 4-fold in the Δ*cusR*MG pVS198 sample. Only *rrsD*, *rrsA*, *rrsC* and *yedY* are uniquely regulated in Δ*cusRS*MG. Among the genes affected in the knockout strains are for instance *met* genes of the methionine biosynthesis, also several ribosomal RNA and transporter genes are differentially regulated.

Like also indicated in the scatter plots *hiuH*, *yedV* and *yedW* are upregulated in the Δ*cusRS*MG but downregulated in the Δ*cusR*MG strain. A list showing differential gene expression between Δ*cusR*MG and Δ*cusRS*MG is depicted in Table 3.3.4 which also shows that three more genes *yedY*, *yedZ* and *csgB* are regulated in a different manner between the strains and therefore might also be influenced by CusS HK. Possible is a phosphatase activity of CusS on the regulator which is responsible for a slight upregulation of the genes in a CusR-independent manner.

Table 3.3.4 Differential gene expression between Δ*cusR*MG pVS198 and Δ*cusRS*MG pVS198.

Gene	Annotation	Δ*cusR*MG pVS198 → Δ*cusRS*MG pVS198
yedV	putative sensory kinase in two-component regulatory system with YedW	204.8
yedW	putative DNA-binding response regulator in two-component system with YedV	173.6
hiuH	hydroxyisourate hydrolase	51.8
yedY	membrane-anchored, periplasmic TMAO, DMSO reductase	8.1
yedZ	inner membrane heme subunit for periplasmic YedYZ reductase	6.9
csgB	curlin nucleator protein, minor subunit in curli complex	-4.7

3.3.1.3 Effect of CusR on gene expression

CusR overexpression in MG1655 was performed to identify copper stimulus-independent gene regulation by the CusS/ CusR TCS.

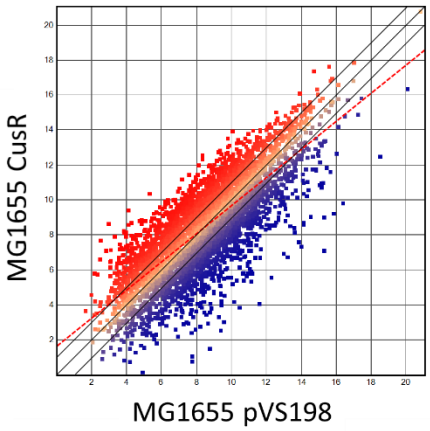

Figure 3.3.3 Scatter plot of gene expression between MG1655 pVS198 and MG1655 CusR

As seen in the scatter plot in Figure 3.3.3 overexpression of CusR affects a multitude of genes. The number of affected genes might be extended due to a linear correlation of only R^2=0.6234, an indication that the sample quality is not optimal. Therefore only the 15 most up- or downregulated genes are depicted in Table 3.3.5. Genes with a fold change greater than 10 are depicted in Table 7.1.12 and fold changes of 4 to 10 are depicted in Table 7.1.7 in the Appendix.

Table 3.3.5 Genes with highest fold change between MG1655 pVS198 and MG1655 CusR

Gene	Annotation	MG1655 CusR
yjiC		52.2
ilvM	acetolactate synthase II, small subunit	39.5
iraM	RpoS stabilizer during Mg starvation, anti-RssB factor	36.6
yjfZ		32.6
yeaI	putative membrane-anchored diguanylate cyclase	24.1
yhcA	putative periplasmic chaperone protein	22.6
yghF		22.0
cusC	copper/silver efflux system, outer membrane component	21.6
ybbD		19.7
ilvL	ilvG operon leader peptide	19.5
ydcC		19.2
yafT	Lipoprotein	18.0
gfcB	putative outer membrane lipoprotein	18.0
arpB		17.4
rrsD	16S ribosomal RNA of rrnD operon	16.6
yebA	putative peptidase	-74.7
flu	CP4-44 prophage, antigen 43 (Ag43) phase-variable biofilm formation autotransporter	-76.4
zinT	zinc and cadmium binding protein, periplasmic	-88.6
msrB	methionine sulfoxide reductase B	-91.6
ykgM	50S ribosomal protein L31 type B, alternative L31 utilized during zinc limitation	-95.6
glpK	glycerol kinase	-96.2
gapA	glyceraldehyde-3-phosphate dehydrogenase A	-97.9
cyoC	cytochrome o ubiquinol oxidase subunit III	-100.1
yncE	ATP-binding protein, periplasmic, function unknown	-100.7
ykgO	RpmJ-like protein	-113.7
cyoD	cytochrome o ubiquinol oxidase subunit IV	-184.3
znuA	zinc transporter subunit: periplasmic-binding component of ABC superfamily	-295.3

Upon overexpression of CusR in the MG1655 wildtype many genes show a positive fold change in comparison with the wildtype carrying an empty plasmid as a control. Among the upregulated genes is for instance *cusC*, which is part of the CusCFBA copper exporter and described to be regulated by the CusS/ CusR TCS. Other genes upregulated are transporters, transcriptional regulators, chaperons and lipoproteins. Also the HK TorS exhibits a positive fold change of 8.9 upon CusR overexpression. A downregulation can be observed for genes encoding for the flagellin and curli subunits *fliC* and *csgA*. Also downregulated are parts of the respiratory chain, genes involved in the murein biosynthesis and the *zinT* and *znuA* genes which are involved in the response to zinc and were also seen to be affected in the copper response experiment in

BW25113. CusR overexpression seems to have an effect on the expression of the RR YedW, which is downregulated 8.7-fold compared to the wildtype.

The overexpression of CusR should in general activate all genes regulated by the CusS/ CusR TCS. To validate the genes found to be CusR dependent in Δ*cusR*$_{BW}$ upon copper stimulation two different gene sets are compared (Figure 3.3.4 and Table 3.3.6). The gene set MG1655 CusR comprises all CusR regulated genes, the gene set [(BW25113 -/+ $CuSO_4$) – (Δ*cusR*$_{BW}$ -/+ $CuSO_4$)] contains genes upregulated by copper stimulus only if the CusR is present.

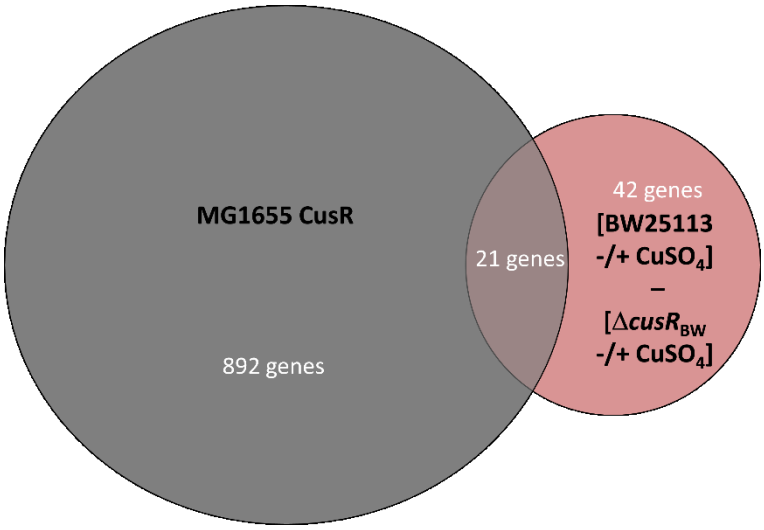

Figure 3.3.4 Venn diagram of gene sets regulated more than 4-fold in MG1655 CusR and genes upregulated by copper stimulus only if the CusR is present in BW25113.

Table 3.3.6 Intersecting set of Venn diagram (see above). Genes more than 4-fold regulated in [BW25113 -/+ $CuSO_4$] – [Δ*cusR*$_{BW}$ -/+ $CuSO_4$].

Gene	Annotation	BW25113 → BW25113 $CuSO_4$	MG1655 pVS198 → MG1655 CusR
cusC	copper/silver efflux system, outer membrane component	305.7	21.6
cusB	copper/silver efflux system, membrane fusion protein	177.6	4.7
ykgO	RpmJ-like protein	20.5	-113.7
hiuH	hydroxyisourate hydrolase	14.9	-11.5
chaA	calcium/sodium:proton antiporter	7.4	-4.3
rrsA	16S ribosomal RNA of rrnA operon	5.7	9.1
ibpA	heat shock chaperone	5.2	5.5
ygiM	SH3 domain protein	5.0	-34.4

Gene	Annotation	BW25113 → BW25113 $CuSO_4$	MG1655 pVS198 → MG1655 CusR
ibpB	heat shock chaperone	4.3	10.7
yrbN		4.3	4.9
pmrR	putative regulator of BasS activity, membrane protein	4.2	4.6
ydeH	diguanylate cyclase, required for pgaD induction	4.1	6.4
rrsC	16S ribosomal RNA of rrnC operon	4.0	8.7
znuC	zinc transporter subunit: ATP-binding component of ABC superfamily	4.0	-30.3
osmY	periplasmic protein	-4.0	-10.5
yjiC		-4.0	52.2
lamB	maltose outer membrane porin (maltoporin)	-4.0	-5.5
ytjA		-4.1	-42.8
zntA	zinc, cobalt and lead efflux system	-5.2	4.6
fliC	flagellar filament structural protein (flagellin)	-5.9	-13.1
hdeD	acid-resistance membrane protein	-5.9	-8.5

The Venn diagram identifies, amongst others, *cusC* to be upregulated under copper-stimulating conditions and upon overexpression of the CusR RR. The upregulation is more conspicuous in the copper stimulated sample, which might be due to an increase of kinase activity of the corresponding HK. Several genes show an increased expression upon copper stimulation and a decreased expression upon overexpression of CusR or vice versa. Among them are the predicted signal transduction protein YgiM, the ribosomal protein paralog YkgO and HiuH, a 5-hydroxyisourate hydrolase. Expression of these genes is low in Δ*cusR*$_{MG}$ and might be repressed by higher amounts of CusR, a repression which is relieved upon copper stimulation.

3.3.2 Gene regulation by the BaeS/ BaeR TCS

The BaeS/ BaeR TCS signaling is together with other signalling pathways involved in the envelope stress response. It is triggered by indole, the overexpression of the pilin subunit PapG and more recently also sodium-tungstate and flavenoids have been described to activate BaeS/ BaeR [215]. BaeS/ BaeR is involved in the expression of the multidrug exporter genes *mdtABCD* and *arcD* genes as well as *spy* [17], [19]. Other genes like *phoB*, *phoR*, *tolC*, *fliACDST*, *motAB*, members of the *che* and maltose operone are also affected by BaeR overexpression [19]. The Δ*baeSR* knockout affects the regulation of several genes for instance *mdtABCD*, *cheR*, *cheZ*, *acrD*, *spy* and others.

As our collaborator observed positive FRET interaction upon stimulation with 30 µM copper for the BaeS HK [216] I investigated the effect of copper on the signaling in Δ*baeR*$_{BW}$. When comparing the RNA sample of the BW25113 wildtype with the ones from the previously discussed Δ*cusR*$_{BW}$ and the Δ*baeR*$_{BW}$ strain a strong upregulation of motility, flagellar and chemotaxis genes can be observed in both knockout strains. This cannot be observed in the Δ*yedW*$_{BW}$ knockout strain, which will be discussed in Chapter 3.3.3. Due to this one can assume that there are some discrepancies in the regulation of these genes in different strains of the Keio collection [194]. To avoid false positive matches I therefore looked at the copper effect in Δ*baeR*$_{BW}$ compared to Δ*cusR*$_{BW}$ instead of comparing it to the wildtype. Genes which are affected more than 4-fold in Δ*baeR*$_{BW}$ without and with copper stimulus are comprised in Table 3.3.7 and 3.3.8.

Table 3.3.7 Genes with fold change more than 4-fold between Δ*cusR*$_{BW}$ and Δ*baeR*$_{BW}$

Gene	Annotation	Δ*cusR*$_{BW}$ → Δ*baeR*$_{BW}$	Δ*cusR*$_{BW}$ $CuSO_4$ → Δ*baeR*$_{BW}$ $CuSO_4$
cusR	DNA-binding response regulator in two-component regulatory system with CusS	140.0	14372.3
argF	ornithine carbamoyltransferase 2, chain F; CP4-6 prophage	95.9	-20.2
argI	ornithine carbamoyltransferase 1	93.1	-47.6
artJ	arginine binding protein, periplasmic	64.3	-26.5
argA	fused acetylglutamate kinase homolog (inactive)/amino acid N-acetyltransferase	59.5	-13.1
argC	N-acetyl-gamma-glutamylphosphate reductase, NAD(P)-binding	59.2	-15.3
argB	acetylglutamate kinase	26.6	-13.7
argG	argininosuccinate synthetase	21.7	-14.9
efeU		20.0	2.0
argD	bifunctional acetylornithine aminotransferase/ succinyldiaminopimelate aminotransferase	16.6	-6.9
azuC		11.5	-4.8
ilvX		10.9	-2.2
pyrI	aspartate carbamoyltransferase, regulatory subunit	10.3	-1.4
ilvG		9.5	-5.7
fimA	major type 1 subunit fimbrin (pilin)	9.4	5.7
alaE	alanine exporter, alanine-inducible, stress-responsive	9.1	-12.0
argH	argininosuccinate lyase	8.7	-10.0
argE	acetylornithine deacetylase	7.8	-8.0
pyrB	aspartate carbamoyltransferase, catalytic subunit	7.5	-1.2
efeO	inactive ferrous ion transporter EfeUOB	6.5	2.2
yagU	inner membrane protein, DUF1440 family	6.1	1.5
yagI	CP4-6 prophage, predicted DNA-binding transcriptional	5.3	-4.6

Gene	Annotation	Δ*cusR*$_{BW}$ → Δ*baeR*$_{BW}$	Δ*cusR*$_{BW}$ $CuSO_4$ → Δ*baeR*$_{BW}$ $CuSO_4$
	regulator		
ilvC	ketol-acid reductoisomerase, NAD(P)-binding	4.5	1.4
trxC	thioredoxin 2	4.2	-2.8
psiE	phosphate starvation inducible protein	4.1	-7.2
ilvB	acetolactate synthase I, large subunit	4.0	-3.6
gtrB	CPS-53 (KpLE1) prophage, bactoprenol glucosyl transferase	-4.0	7.3
tpiA	triosephosphate isomerase	-4.0	3.5
mzrA	modulator of EnvZ/OmpR regulon	-4.0	-2.3
yceI	secreted protein	-4.0	-2.6
yliI	soluble aldose sugar dehydrogenase	-4.1	1.7
oppB	oligopeptide transporter subunit	-4.1	8.1
ygiC	glutathionylspermidine synthase homolog	-4.1	1.2
fabA	beta-hydroxydecanoyl thioester dehydrase	-4.1	2.9
tsaB	tRNA(ANN) t(6)A37 threonylcarbamoyladenosine modification protein, binding partner and protease for TsaD	-4.1	5.0
degP	serine endoprotease (protease Do), membrane-associated	-4.2	-1.1
metT	tRNA-Met	-4.2	14.4
metU	tRNA-Met	-4.2	14.4
tyrT	tRNA-Tyr	-4.3	2.7
tyrV	tRNA-Tyr	-4.3	2.7
cysW	sulfate/thiosulfate ABC transporter subunit	-4.4	-1.3
nusB	transcription antitermination protein	-4.5	4.7
cpxA	sensory histidine kinase in two-component regulatory system with CpxR	-4.7	2.2
nupC	nucleoside (except guanosine) transporter	-4.7	3.6
fliQ	flagellar biosynthesis protein	-4.9	7.4
ydeH	diguanylate cyclase, required for pgaD induction	-5.0	-5.6
ribE	riboflavin synthase beta chain	-5.1	3.7
purE	N5-carboxyaminoimidazole ribonucleotide mutase	-5.1	11.9
ymfE	e14 prophage, predicted inner membrane protein	-5.2	5.3
nmpC		-5.3	2.6
folK	2-amino-4-hydroxy-6-hydroxymethyldihyropteridine pyrophosphokinase	-5.4	4.3
cysI	sulfite reductase, beta subunit, NAD(P)-binding, heme-binding	-5.5	1.3
cysH	3'-phosphoadenosine 5'-phosphosulfate reductase	-5.9	1.8
yeeN	conserved protein, UPF0082 family	-6.1	13.7
chaA	calcium/sodium:proton antiporter	-6.5	-2.9
cyoA	cytochrome o ubiquinol oxidase subunit II	-7.2	-3.5
yccA	HflBKC-binding inner membrane protein, UPF0005 family	-7.7	1.5
tnaC	tryptophanase leader peptide	-7.8	19.5
yebE	inner membrane protein, DUF533 family	-8.7	-11.2
yncJ		-9.5	-3.2
yobB		-9.9	-3.1
yoeI		-10.4	28.0

Gene	Annotation	Δ*cusR*$_{BW}$ → Δ*baeR*$_{BW}$	Δ*cusR*$_{BW}$ $CuSO_4$ → Δ*baeR*$_{BW}$ $CuSO_4$
cpxP	inhibitor of the cpx response, periplasmic adaptor protein	-12.1	-2.9
gtrS	serotype-specific glucosyl transferase, CPS-53 (KpLE1) prophage	-13.5	16.0
cyoC	cytochrome o ubiquinol oxidase subunit III	-13.6	-14.0
cyoE	protoheme IX farnesyltransferase	-13.7	-10.6
yhdU	putative membrane protein	-13.7	21.6
ycfS	L,D-transpeptidase linking Lpp to murein	-14.7	-1.5
cyoD	cytochrome o ubiquinol oxidase subunit IV	-17.0	-12.6
cyoB	cytochrome o ubiquinol oxidase subunit I	-27.2	-18.2

The gene expression in Δ*cusR*$_{BW}$ → Δ*baeR*$_{BW}$ without a copper stimulus show an upregulation of many *arg* genes which are involved in the biosynthesis of arginine. Also for instance the pilin subunit FimA is upregulated but over all not that many genes are positively affected in Δ*cusR*$_{BW}$ → Δ*baeR*$_{BW}$. Downregulation can be seen for genes belonging to the *cyo* family which are involved in the respiratory chain in the membrane. Also the gene expression of the TCS CpxA/ CpxR, which is also involved in membrane stress signaling, is affected in Δ*baeR*$_{BW}$. Interestingly none of the genes described to be regulated by the BaeS/ BaeR TCS are affected in the non-stimulated Δ*baeR*$_{BW}$ sample.
I also looked at how copper changes the gene expression profile in Δ*baeR*$_{BW}$ to see whether the lack of signaling by the BaeS/ BaeR TCS has an impact on genes regulated upon exposure to copper. Affected genes are comprised in Table 3.3.8.

Table 3.3.8 Genes with changes more than 4-fold between Δ*cusR*$_{BW}$ $CuSO_4$ and Δ*baeR*$_{BW}$ $CuSO_4$

Gene	Annotation	Δ*cusR*$_{BW}$ → Δ*baeR*$_{BW}$	Δ*cusR*$_{BW}$ $CuSO_4$ → Δ*baeR*$_{BW}$ $CuSO_4$
cusR	DNA-binding response regulator in two-component regulatory system with CusS	140.0	14372.3
cusF	periplasmic copper- and silver-binding protein	1.3	852.2
cusC	copper/silver efflux system, outer membrane component	1.3	389.3
cusB	copper/silver efflux system, membrane fusion protein	1.3	176.0
yoeI		-10.4	28.0
hiuH	hydroxyisourate hydrolase	-1.3	27.6
yhdU	putative membrane protein	-13.7	21.6
tnaC	tryptophanase leader peptide	-7.8	19.5
cusA	copper/silver efflux system, membrane component	1.5	18.1

Gene	Annotation	Δ*cusR*$_{BW}$ → Δ*baeR*$_{BW}$	Δ*cusR*$_{BW}$ $CuSO_4$ → Δ*baeR*$_{BW}$ $CuSO_4$
cspA	RNA chaperone and anti-terminator, cold-inducible	-1.4	17.4
gtrS	serotype-specific glucosyl transferase, CPS-53 (KpLE1) prophage	-13.5	16.0
yoaK	expressed protein, membrane-associated	-1.8	15.4
metT	tRNA-Met	-4.2	14.4
metU	tRNA-Met	-4.2	14.4
yeeN	conserved protein, UPF0082 family	-6.1	13.7
fliN	flagellar motor switching and energizing component	-1.5	13.0
purE	N5-carboxyaminoimidazole ribonucleotide mutase	-5.1	11.9
ileT	tRNA-Ile	-3.3	11.7
ileU	tRNA-Ile	-3.3	11.7
ileV	tRNA-Ile	-3.3	11.7
fliO	flagellar biosynthesis protein	-1.8	11.4
zraP	Zn-dependent periplasmic chaperone	-1.4	10.4
rrsA	16S ribosomal RNA of rrnA operon	-2.9	10.4
cheY	chemotaxis regulator transmitting signal to flagellar motor component	-1.5	10.2
ndk	multifunctional nucleoside diphosphate kinase and apyrimidinic endonuclease and 3'-phosphodiesterase	-2.8	10.1
yahO	periplasmic protein, function unknown, YhcN family	1.1	10.0
trpL	trp operon leader peptide	-1.4	9.9
lysV	tRNA-Lys	-2.9	9.8
lysQ	tRNA-Lys	-2.9	9.8
lysZ	tRNA-Lys	-2.9	9.8
lysY	tRNA-Lys	-2.9	9.8
lysW	tRNA-Lys	-2.9	9.8
lysT	tRNA-Lys	-2.9	9.8
rbsD	putative cytoplasmic sugar-binding protein	-1.6	9.6
yeaQ	conserved protein, UPF0410 family	-1.2	9.3
yecF	conserved protein, DUF2594 family	-1.4	9.3
cusS	sensory histidine kinase in two-component regulatory system with CusR, senses copper ions	1.2	9.0
flgH	flagellar protein of basal-body outer-membrane L ring	1.2	8.8
rrsC	16S ribosomal RNA of rrnC operon	-2.2	8.7
glpT	sn-glycerol-3-phosphate transporter	-1.6	8.5
flhD	DNA-binding transcriptional dual regulator with FlhC	-3.3	8.5
fliG	flagellar motor switching and energizing component	-1.3	8.4
yoeH		1.6	8.3
yciH	initiation factor function partial mimic, SUI1 family	-2.8	8.2
oppB	oligopeptide transporter subunit	-4.1	8.1
yecT		1.2	8.0
argE	acetylornithine deacetylase	7.8	-8.0
fdrA	multicopy suppressor of dominant negative ftsH mutations, predicted acyl-CoA synthetase with NAD(P)-binding Rossmann-fold domain	1.4	-8.3
kdpA	potassium translocating ATPase, subunit A	-1.2	-8.3

Gene	Annotation	Δ*cusR*$_{BW}$ → Δ*baeR*$_{BW}$	Δ*cusR*$_{BW}$ $CuSO_4$ → Δ*baeR*$_{BW}$ $CuSO_4$
rhsD	rhsD element protein	1.2	-8.5
hyfD	hydrogenase 4, membrane subunit	1.4	-8.5
yiaN	L-dehydroascorbate transporter, TRAP permease large subunit for TRAP (TRipartite ATP-independent Periplasmic) family transporter YiaMNO	1.2	-8.7
yphG		1.6	-8.8
eutJ	putative chaperonin, ethanolamine utilization protein	1.3	-9.4
yhfX	putative amino acid racemase	1.5	-9.5
nikC	nickel transporter subunit	1.0	-9.8
yiiE	putative transcriptional regulator	3.4	-9.9
hycC	hydrogenase 3, membrane subunit	1.3	-9.9
argH	argininosuccinate lyase	8.7	-10.0
rhsA	rhsA element core protein RshA	1.4	-10.0
nrfD	formate-dependent nitrite reductase, membrane subunit	1.1	-10.2
mhpA	3-(3-hydroxyphenyl)propionate hydroxylase	1.2	-10.3
hycD	hydrogenase 3, membrane subunit	1.1	-10.6
cyoE	protoheme IX farnesyltransferase	-13.7	-10.6
mngA	fused 2-O-a-mannosyl-D-glycerate specific PTS enzymes: IIA component/IIB component/IIC component	1.7	-10.9
eutS	putative carboxysome structural protein with predicted role in ethanol utilization	-1.1	-11.1
acrD	aminoglycoside/multidrug efflux system	-1.6	-11.2
yebE	inner membrane protein, DUF533 family	-8.7	-11.2
phnK	carbon-phosphorus lyase complex subunit, putative ATP transporter ATP-binding protein	1.6	-11.4
lyxK	L-xylulose kinase	1.5	-11.4
alaE	alanine exporter, alanine-inducible, stress-responsive	9.1	-12.0
rhsB	rhsB element core protein RshB	1.2	-12.2
cyoD	cytochrome o ubiquinol oxidase subunit IV	-17.0	-12.6
argA	fused acetylglutamate kinase homolog (inactive)/amino acid N-acetyltransferase	59.5	-13.1
argB	acetylglutamate kinase	26.6	-13.7
spy	periplasmic ATP-independent protein refolding chaperone, stress-induced	-3.9	-14.0
cyoC	cytochrome o ubiquinol oxidase subunit III	-13.6	-14.0
argG	argininosuccinate synthetase	21.7	-14.9
argC	N-acetyl-gamma-glutamylphosphate reductase, NAD(P)-binding	59.2	-15.3
cyoB	cytochrome o ubiquinol oxidase subunit I	-27.2	-18.2
phnI	ribophosphonate triphosphate synthase complex, probable catalytic subunit	1.5	-19.5
argF	ornithine carbamoyltransferase 2, chain F; CP4-6 prophage	95.9	-20.2
yjfN		-3.2	-20.8
artJ	arginine binding protein, periplasmic	64.3	-26.5
argI	ornithine carbamoyltransferase 1	93.1	-47.6

In Δ*baeR*$_{BW}$ the copper stimulus induces the genes identified to be copper-responsive and regulated by the CusR like the *cusC* operon and *hiuH*. As I was looking for genes which are regulated by the BaeS/ BaeR TCS in a copper dependent manner, genes with a negative fold change are candidates as the activation of genes of interest would be impaired in Δ*baeR*$_{BW}$. A similar downregulatory effect in Δ*cusR*$_{BW}$ $CuSO_4$ → Δ*baeR*$_{BW}$ $CuSO_4$ can be observed. Downregulation like in the unstimulated sample can be seen for the *cyo* genes and does therefore not depend on copper regulation.
To describe which genes are uniquely regulated by BaeR upon copper stimulation gene regulation in Δ*cusR*$_{BW}$ to Δ*baeR*$_{BW}$ is compared (Figure 3.3.7) and genes only regulated when BaeR is present in the cell are identified (Table 3.3.9).

Figure 3.3.7 Venn diagram of gene sets regulated more than 4-fold upon stimulation with 1 mM copper in Δ*cusR*$_{BW}$ and Δ*baeR*$_{BW}$.
(linear correlation coefficient in Table 7.1.6 in the Appendix)

Table 3.3.9 Gene set of Venn diagram (see above). Genes more than 4-fold regulated in Δ*cusR*$_{BW}$ -/+ $CuSO_4$ but not in Δ*baeR*$_{BW}$ -/+ $CuSO_4$.

Gene	Annotation	Δ*cusR*$_{BW}$ → Δ*cusR*$_{BW}$ $CuSO_4$	Δ*baeR*$_{BW}$ → Δ*baeR*$_{BW}$ $CuSO_4$
argI	ornithine carbamoyltransferase 1	165.7	-26.8
argF	ornithine carbamoyltransferase 2, chain F; CP4-6 prophage	83.1	-23.3
artJ	arginine binding protein, periplasmic	74.3	-22.9
argC	N-acetyl-gamma-glutamylphosphate reductase, NAD(P)-binding	54.9	-16.5
argA	fused acetylglutamate kinase homolog (inactive)/amino acid N-	52.7	-14.8

Gene	Annotation	Δ*cusR*$_{BW}$ → Δ*cusR*$_{BW}$ $CuSO_4$	Δ*baeR*$_{BW}$ → Δ*baeR*$_{BW}$ $CuSO_4$
	acetyltransferase		
argG	argininosuccinate synthetase	40.0	-8.1
argB	acetylglutamate kinase	38.4	-9.5
argH	argininosuccinate lyase	26.5	-3.3
argD	bifunctional acetylornithine aminotransferase/ succinyldiaminopimelate aminotransferase	16.8	-6.8
yiiE	putative transcriptional regulator	11.6	-2.9
argE	acetylornithine deacetylase	11.4	-5.5
alaE	alanine exporter, alanine-inducible, stress-responsive	8.4	-13.0
cheR	chemotaxis regulator, protein-glutamate methyltransferase	-8.0	1.2
ypdK	expressed protein, membrane-associated	-8.3	1.8
flhD	DNA-binding transcriptional dual regulator with FlhC	-8.8	3.2
oppB	oligopeptide transporter subunit	-9.2	3.6
metU	tRNA-Met	-9.4	6.5
metT	tRNA-Met	-9.4	6.5
purE	N5-carboxyaminoimidazole ribonucleotide mutase	-9.7	6.3
yhdU	putative membrane protein	-12.7	23.4
yeeN	conserved protein, UPF0082 family	-12.8	6.5
tnaC	tryptophanase leader peptide	-18.5	8.3
gtrS	serotype-specific glucosyl transferase, CPS-53 (KpLE1) prophage	-18.7	11.5
yoeI		-19.7	14.8

A majority of upregulated genes in Δ*cusR*$_{BW}$ upon stimulation with copper are involved in the arginine biosynthesis. As described above this is due to the lack of components required for copper homeostasis in the cell [178]. The Δ*baeR*$_{BW}$ is not defective for these components in turn arginine biosynthesis genes are observed to be downregulated. Aside from the arginine biosynthesis only two more genes are upregulated. The alanine exporter gene *alaE* and *yiiE* which encodes for a putative transcriptional regulator of unknown function are upregulated upon copper stimulation. Downregulation is for instance observed for genes involved in regulation of motility and chemotaxis (*flhD* and *cheR*). The strongest downregulation can be observed for *yoeI*, a gene of unknown function. Overall only a few non-associated genes are identified in the comparison of Δ*cusR*$_{BW}$ -/+ $CuSO_4$ with ΔbaeR$_{BW}$ -/+ $CuSO_4$. On the level of gene expression there is no clear evidence for an effect of copper on the signaling of the BaeS/ BaeR TCS.

3.3.3 Gene regulation by the YedV/ YedW TCS

As YedV/ YedW has been shown to be closely connected to the CusS/ CusR TCS but its function had not been resolved I was interested in identifying and studying target genes of this TCS. In order to do so RNA deep sequencing in Δ*yedW*$_{BW}$ and in MG1655 strains with overexpressed components of the YedV/ YedW operon was performed.

The effect of Δ*yedW*$_{BW}$ in comparison with the BW25113 was used to search for genes which are commonly regulated by YedW. The Δ*yedW*$_{BW}$ experiment was also performed with addition of a $CuSO_4$ as stimulus, which activates CusS/ CusR signaling. This was done to clarify whether the absence YedW RR affects the copper stimulon and whether YedV/ YedW is involved in copper sensing (Figure 3.3.8).

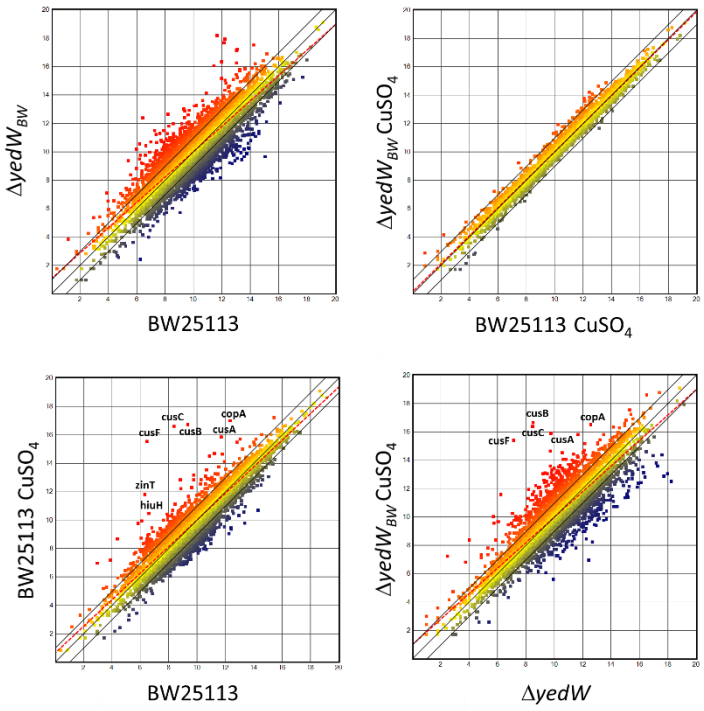

Figure 3.3.8 Scatter plots fold changes between BW25113 wildtype and Δ*yedW*$_{BW}$ with or without $CuSO_4$ stimulus.

The scatter plots show genes which are differentially regulated either in the BW25113 wildtype or the Δ*yedW*$_{BW}$ strain and also compare the strains to each other under non-

and stimulated conditions. These genes are summarized in Table 3.3.10. Genes with a negative fold change in Δ*yedW*$_{BW}$ are potential candidates for positive regulation by YedW. The addition of a copper stimulus is not affected by the lack of YedW as the gene expression pattern in BW25113 $CuSO_4$→ Δ*yedW*$_{BW}$ $CuSO_4$ shows no distinctly up- or downregulated genes. This is further supported by the scatter plots comparing the effect of copper on gene expression in BW25113 → BW25113 $CuSO_4$ and Δ*yedW*$_{BW}$ → Δ*yedW*$_{BW}$ $CuSO_4$. The expression profiles of genes like *cusC* which are upregulated upon $CuSO_4$ stimulation in the wildtype are similar to the expression profiles of genes in Δ*yedW*$_{BW}$. Table 3.3.10 depicts all genes with a fold change of more than 8 in BW25113→ Δ*yedW*$_{BW}$ as well as 'genes of interest' which are known to be affected by copper or potentially regulated by YedV/ YedW. They are also shown in a heat map of gene expression profiles in Figure 3.3.4. Smaller fold changes from 4 to 8 can be found in Table 7.1.8 in the Appendix.

Table 3.3.10 Genes with fold changes more than 8 and genes of interest between BW25113 and Δ*yedW*$_{BW}$.
Grey: fold change in experiments with $CuSO_4$ and light grey: fold change between experiments without and with $CuSO_4$.

Gene	Annotation	BW25113 → Δ*yedW*$_{BW}$	BW25113 $CuSO_4$ → Δ*yedW*$_{BW}$ $CuSO_4$	BW25113 $CuSO_4$ → BW25113 $CuSO_4$	Δ*yedW*$_{BW}$ $CuSO_4$ → Δ*yedW*$_{BW}$ $CuSO_4$
glpT	sn-glycerol-3-phosphate transporter	-39.5	3.0	-3.8	31.5
dppB	dipeptide/heme transporter	-15.1	-1.0	-1.2	12.2
mglB	methyl-galactoside transporter subunit	-14.5	2.8	-2.7	15.0
nmpC		-14.2	1.5	-2.5	8.4
tnaC	tryptophanase leader peptide	-13.9	2.9	-1.4	28.4
mglA	fused methyl-galactoside transporter subunits of ABC superfamily: ATP-binding components	-13.0	3.4	-3.1	14.0
oppC	oligopeptide transporter subunit	-12.6	-1.1	-1.5	8.0
dppD	dipeptide/heme transporter	-11.6	1.0	-1.3	9.5
purK	N5-carboxyaminoimidazole ribonucleotide synthase	-11.4	1.3	1.0	14.5
oppB	oligopeptide transporter subunit	-11.3	-1.1	-1.6	6.5
dctA	C4-dicarboxylic acid, orotate and citrate transporter	-11.2	2.3	-3.8	6.9
thiH	tyrosine lyase, involved in thiamin-thiazole moiety synthesis	-10.5	1.1	-1.0	11.4
dppC	dipeptide/heme transporter	-10.3	1.0	-1.2	8.5
murF	UDP-N-acetylmuramoyl-tripeptide:D-alanyl-D-	-9.8	-1.1	1.2	10.6

Gene	Annotation	BW25113 → Δ*yedW*$_{BW}$	BW25113 $CuSO_4$ → Δ*yedW*$_{BW}$ $CuSO_4$	BW25113 → BW25113 $CuSO_4$	Δ*yedW*$_{BW}$ → Δ*yedW*$_{BW}$ $CuSO_4$
gtrS	alanine ligase serotype-specific glucosyl transferase, CPS-53 (KpLE1) prophage	-9.7	-1.2	1.8	14.7
oppD	oligopeptide transporter subunit	-9.7	-1.1	-1.4	6.2
glpF	glycerol facilitator	-9.4	1.7	-1.8	8.8
ndk	multifunctional nucleoside diphosphate kinase and apyrimidinic endonuclease and 3'-phosphodiesterase	-9.3	2.7	-2.6	9.9
mglC	methyl-galactoside transporter subunit	-9.2	2.7	-2.7	9.3
plaP	putrescine importer, low affinity	-9.2	1.9	-1.6	11.0
aspA	aspartate ammonia-lyase	-8.7	2.1	-3.3	5.5
yjcD	putative permease	-8.3	1.1	-1.0	8.7
sdaB	L-serine deaminase II	-8.2	1.9	-4.0	4.0
suhB	inositol monophosphatase	-8.2	2.4	-1.9	10.2
mraY	phospho-N-acetylmuramoyl-pentapeptide transferase	-8.2	-1.0	1.2	9.2
rof	modulator of Rho-dependent transcription termination	8.3	-1.4	1.7	-6.8
mqsR	GCU-specific mRNA interferase toxin of the MqsR-MqsA toxin-antitoxin system, biofilm/motility regulator, anti-repressor	8.4	-1.1	2.5	-3.8
yagI	CP4-6 prophage, predicted DNA-binding transcriptional regulator	8.4	1.2	1.4	-5.0
ykiA		8.5	-1.4	2.0	-5.9
pspB	DNA-binding transcriptional regulator of psp operon	8.6	-1.0	5.0	-1.8
yciY		8.7	-1.0	2.7	-3.3
yhfG	putative Fic-binding protein	8.8	-1.2	1.7	-6.1
hisL	his operon leader peptide	8.9	-1.2	4.5	-2.3
ydcY		9.2	-1.3	1.9	-6.6
uspB	universal stress (ethanol tolerance) protein B	9.4	-1.6	1.3	-11.3
pspG	phage shock protein G	9.4	-1.0	5.6	-1.7
cspI	Qin prophage, cold shock protein	9.6	-1.9	10.2	-1.7
gnsA	multicopy suppressor of secG(Cs) and fabA6(Ts), predicted regulator of phosphatidylethanolamine synthesis	9.6	-1.4	3.1	-4.2
argE	acetylornithine deacetylase	9.7	1.4	1.2	-5.7
ilvG		9.7	-1.4	1.5	-8.9
uspG	universal stress protein UP12	9.8	-1.2	1.1	-10.8
rrfA	5S ribosomal RNA of rrnA operon	9.9	1.6	2.7	-2.3
argG	argininosuccinate synthetase	10.2	2.2	-1.9	-9.0
bssS	biofilm regulator	11.2	-1.2	1.7	-8.1
trxC	thioredoxin 2	11.5	1.0	1.7	-6.5
torI	response regulator inhibitor for tor operon	11.8	-1.2	3.2	-4.6

Gene	Annotation	BW25113 → Δ*yedW*$_{BW}$	BW25113 $CuSO_4$ → Δ*yedW*$_{BW}$ $CuSO_4$	BW25113 $CuSO_4$ → BW25113 $CuSO_4$	Δ*yedW*$_{BW}$ $CuSO_4$ → Δ*yedW*$_{BW}$ $CuSO_4$
yneM	expressed protein, membrane-associated	12.4	-1.0	4.5	-2.8
yhcN		12.4	-1.5	5.0	-3.8
mqsA	antitoxin for MqsR toxin, transcriptional repressor	12.5	-1.3	2.4	-6.6
bhsA	biofilm, cell surface and signaling protein	13.2	-1.6	2.7	-7.8
yibT		13.3	-1.6	3.2	-6.5
ybfA		13.5	-1.5	2.2	-9.3
ilvM	acetolactate synthase II, small subunit	14.0	-1.3	3.5	-5.3
yjcB		15.3	-1.5	2.4	-9.2
cspG	cold shock protein homolog, cold-inducible	16.1	-1.5	2.4	-9.9
argH	argininosuccinate lyase	17.6	1.6	1.1	-10.3
argA	fused acetylglutamate kinase homolog (inactive)/amino acid N-acetyltransferase	18.0	2.3	-1.5	-12.3
cspB	Qin prophage, cold shock protein	19.4	-1.6	3.9	-7.8
psiE	phosphate starvation inducible protein	19.5	-1.6	2.3	-13.2
ilvX		20.9	-1.7	4.5	-7.9
argC	N-acetyl-gamma-glutamylphosphate reductase, NAD(P)-binding	21.4	2.2	-1.2	-11.4
alaE	alanine exporter, alanine-inducible, stress-responsive	23.2	-1.1	1.2	-21.3
argB	acetylglutamate kinase	25.9	2.0	1.1	-12.5
artJ	arginine binding protein, periplasmic	47.1	2.4	-1.4	-27.8
argF	ornithine carbamoyltransferase 2, chain F; CP4-6 prophage	56.5	2.0	-1.1	-30.5
azuC		64.5	-2.0	3.0	-43.5
argI	ornithine carbamoyltransferase 1	98.7	1.9	-1.0	-53.0
	Genes of interest				
cusS	sensory histidine kinase in two-component regulatory system with CusR, senses copper ions	-1.9	1.2	6.1	14.0
cusR	DNA-binding response regulator in two-component regulatory system with CusS	1.1	1.1	11.2	11.6
yedV	putative sensory kinase in two-component regulatory system with YedW	-1.1	-1.5	3.6	2.7
cusC	copper/silver efflux system, outer membrane component	1.0	-1.2	305.7	253.7
hiuH	hydroxyisourate hydrolase	1.5	-1.6	14.9	6.0
yedZ	inner membrane heme subunit for periplasmic YedYZ reductase	1.9	-1.3	2.5	-1.0
yedY	membrane-anchored, periplasmic TMAO, DMSO reductase	1.6	-1.4	1.4	-1.6
cyoA	cytochrome o ubiquinol oxidase subunit II	-2.3	1.4	-1.6	1.9

Among the in BW25113 → Δ*yedW*$_{BW}$ downregulated genes are several which encode for transporter proteins like *glpT*, *oppC* and *dctA*. Genes upregulated are for instance *argI*, *argG* which are involved in the arginine biosynthesis and *azuC* which is a small membrane protein (annotations taken from [198]) and could potentially be negatively regulated by YedW. Genes of interests are also depicted in the heat-map in the Figure 3.3.9 below.

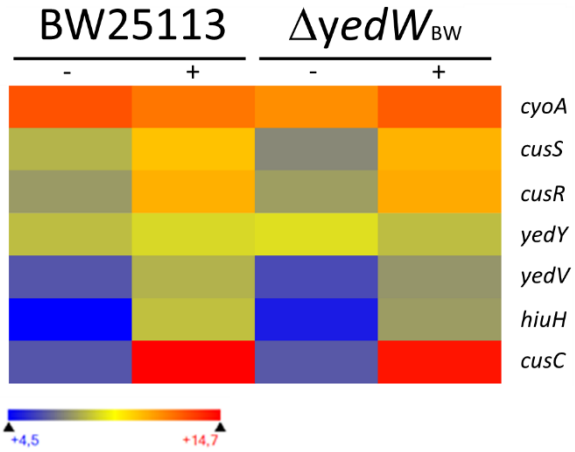

Figure 3.3.9 Heat-map of expression of genes of interest in response to copper.
CusR and potential YedW target genes in *E. coli* strain BW25113 and Δ*yedW*$_{BW}$ knockout strain without and with 1mM copper induction

Genes of interest like *cusC*, *yedV* or *hiuH* show similar fold changes between the wildtype and Δ*yedW*$_{BW}$ with and without stimulus. Also the heat-map indicates that the expression levels are quite similar in the two strains under the same conditions.
To identify whether the genes affected in Δ*yedW*$_{BW}$ are regulated and to identify further genes controlled by YedV/ YedW I performed overexpression experiments in the MG1655 wildtype, in Δ*cusR*$_{MG}$ and in Δ*cusRS*$_{MG}$ strains.

3.3.3.1 YedWV expression

To identify genes which are induced or repressed by an 'active' YedV/ YedW in the MG1655 wildtype YedWV was overexpressed. The same experiment was performed in the Δ*cusR*$_{MG}$ and in Δ*cusRS*$_{MG}$ strains to find possible co-regulation or cross-talk with components of the CusS/ CusR. Scatter plots (Figure 3.3.10) of the wildtype and the

knockout strains show the general gene expression pattern. A Venn diagram identifies genes which are regulated in MG1655 and Δ*cusRS*$_{MG}$ upon YedWV overexpression (Figure 3.3.11). These genes are summarized in Table 3.3.11.

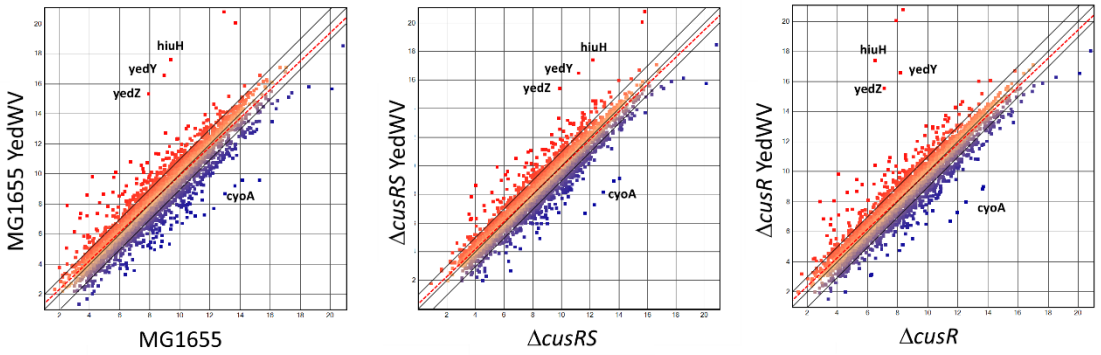

Figure 3.3.10 Scatter plots YedWV expression in MG1655, Δ*cusRS*$_{MG}$ and Δ*cusR*$_{MG}$

Upon overexpression of the YedV/ YedW TCS the scatter plot of gene regulation in the MG1655 wildtype shows three conspicuously upregulated genes and a few intermediately downregulated genes. In addition to the high fold change in *yedV* and *yedW* expression due to induction from a plasmid *hiuH*, *yedY* and *yedZ* exhibit a fold change of 306.6-, 198.3- and 169.8-fold when compared to the wildtype with an empty plasmid. Visibly downregulated genes belong to the *cyo* genes, for instance *cyoA*. Also downregulated is the expression of *fliC*, which encodes for the flagellar subunit flagellin [198]. The genes *flu* and *ompF* are downregulated in the MG1655 experiment, but not in the Δ*cusR*$_{MG}$ and Δ*cusRS*$_{MG}$ experiments. The scatter plots of the Δ*cusR*$_{MG}$ and Δ*cusRS*$_{MG}$ experiments show a similar fold change pattern for the *hiuH*, *yedY*, *YedZ*, *cyo* and *fliC* genes and a downregulation of *metE*, which is involved in the methionine biosynthesis [198]. Gene sets with fold changes more than 4-fold derived from the MG1655 and the Δ*cusRS*$_{MG}$ experiments are depicted in a Venn diagram (Figure 3.3.11).

Figure 3.3.11 Venn diagram of 4-fold regulated genes in MG1655 pVS198→ MG1655 YedWV and Δ*cusRS*$_{MG}$ pVS198→ Δ*cusRS*$_{MG}$ YedWV.

A total of 80 genes are regulated more than 4-fold in response to overexpression of YedWV in both MG1655 wildtype and the Δ*cusRS*$_{MG}$ strain. These are candidates for regulation by YedV/ YedW in a CusS/ CusR-independent manner and are summarized in Table 3.3.11 with their fold changes in all 3 experiments.

Table 3.3.11 Intersecting set of Venn diagram (Figure 3.3.11). Genes both more than 4-fold regulated in MG1655 and Δ*cusRS*$_{MG}$ gene sets. Table sorted by fold changes in MG1655

		YedWV		
Gene	Annotation	MG1655	Δ*cusRS*$_{MG}$	Δ*cusR*$_{MG}$
hiuH	hydroxyisourate hydrolase	306.6	38.5	1994.4
yedW	putative DNA-binding response regulator in two-component system with YedV	240.9	32.6	5654.6
yedY	membrane-anchored, periplasmic TMAO, DMSO reductase	198.3	40.7	343.4
yedZ	inner membrane heme subunit for periplasmic YedYZ reductase	169.8	46.6	356.4
yedV	putative sensory kinase in two-component regulatory system with YedW	85.5	22.5	4608.0
yghW		51.4	22.5	55.8
iraM	RpoS stabilizer during Mg starvation, anti-RssB factor	43.4	18.4	36.8
cusC	copper/silver efflux system, outer membrane component	28.0	24.4	15.6
hybA	hydrogenase 2 4Fe-4S ferredoxin-type component	21.6	10.7	18.8
hybB	putative hydrogenase 2 cytochrome b type component	18.8	10.1	11.7

			YedWV	
Gene	Annotation	MG1655	Δ*cusRS*$_{MG}$	Δ*cusR*$_{MG}$
hybO	hydrogenase 2, small subunit	17.8	13.7	15.6
ecpR	putative regulator	15.9	8.6	14.2
ydeT		14.2	6.4	8.3
ydeS	putative fimbrial-like stabilizing protein	13.5	6.2	5.2
yneM	expressed protein, membrane-associated	13.3	11.9	9.2
ecpB		11.1	8.2	19.6
ydeR	putative fimbrial-like stabilizing protein	10.8	4.9	7.8
mdtJ	multidrug efflux system transporter	9.5	4.4	5.7
cusF	periplasmic copper- and silver-binding protein	9.2	6.4	5.4
ecpA	cryptic Mat fimbrillin gene	9.1	10.2	11.3
ybjG	undecaprenyl pyrophosphate phosphatase	8.0	5.0	5.7
ibpA	heat shock chaperone	7.9	9.5	9.6
ydeQ	putative fimbrial-like stabilizing protein	7.4	4.9	9.9
tqsA	pheromone AI-2 transporter	7.1	6.5	6.9
hybC	hydrogenase 2, large subunit	6.9	5.8	5.2
yodB	cytochrome b561 homolog	6.9	7.2	7.0
rstA	multicopy stabilizer of yjeE, yeaZ or ygjD deletion lethality, predicted response regulator of two-component regulatory system with sensor protein RstB	6.4	7.0	6.5
mgtA	magnesium transporter	6.3	5.2	5.9
cusB	copper/silver efflux system, membrane fusion protein	6.3	5.0	7.1
mgrB	regulatory peptide for PhoPQ, feedback inhibition	6.1	7.6	6.8
ydeH	diguanylate cyclase, required for pgaD induction	6.0	5.7	6.4
rrsD	16S ribosomal RNA of rrnD operon	5.9	5.1	2.6
yebO	putative inner membrane protein	5.8	6.7	6.1
ibpB	heat shock chaperone	5.6	9.2	10.7
maeA	malate dehydrogenase, (decarboxylating, NAD-requiring) (malic enzyme)	5.3	5.3	5.7
ybjX		5.1	4.8	5.9
ecpC	putative aromatic compound dioxygenase	4.9	5.1	5.7
ycjX	conserved protein with nucleoside triphosphate hydrolase domain	4.3	5.0	6.1
rstB	sensory histidine kinase in two-component regulatory system with RstA	4.2	4.2	4.8
slyB	outer membrane lipoprotein	4.0	4.5	5.1
lamB	maltose outer membrane porin (maltoporin)	-4.0	-4.2	-3.5
lhgO	L-2-hydroxyglutarate oxidase	-4.4	-5.0	-3.7
arcB	aerobic respiration control sensor histidine protein kinase, cognate to two-component response regulators ArcA and RssB	-4.4	-4.5	-4.8
treA	periplasmic trehalase	-4.5	-4.1	-3.5
malK	fused maltose transport subunit, ATP-binding component of ABC superfamily/regulatory	-4.6	-5.0	-3.5

			YedWV	
Gene	Annotation	MG1655	Δ*cusRS*$_{MG}$	Δ*cusR*$_{MG}$
	protein			
patD	gamma-aminobutyraldehyde dehydrogenase	-4.6	-4.4	-4.3
hdeD	acid-resistance membrane protein	-4.9	-4.5	-4.7
yagU	inner membrane protein, DUF1440 family	-4.9	-4.6	-3.2
gabD	succinate-semialdehyde dehydrogenase I, NADP-dependent	-4.9	-4.4	-3.9
lacI	DNA-binding transcriptional repressor	-4.9	-4.9	-6.8
gabP	gamma-aminobutyrate transporter	-5.2	-5.9	-4.2
ydcT	putative spermidine/putrescine transporter subunit	-5.3	-4.1	-4.4
otsA	trehalose-6-phosphate synthase	-5.5	-4.4	-5.1
yjdN		-5.5	-5.2	-3.0
gabT	4-aminobutyrate aminotransferase, PLP-dependent	-5.6	-5.1	-4.1
gatD	galactitol-1-phosphate dehydrogenase, Zn-dependent and NAD(P)-binding	-5.6	-4.7	-5.6
csiD	carbon starvation protein	-6.0	-6.1	-4.1
gatC	galactitol-specific enzyme IIC component of PTS	-6.1	-4.3	-5.3
ugpA	glycerol-3-phosphate transporter subunit	-6.7	-13.4	-8.2
otsB	trehalose-6-phosphate phosphatase, biosynthetic	-7.1	-5.9	-6.5
hdeA	stress response protein acid-resistance protein	-7.2	-6.4	-4.9
ycaC	putative hydrolase, isochorismatase family	-7.4	-6.1	-6.8
patA	putrescine:2-oxoglutaric acid aminotransferase, PLP-dependent	-7.4	-5.8	-4.9
yegP	conserved protein, UPF0339 family	-7.5	-5.0	-6.1
aldB	aldehyde dehydrogenase B	-7.9	-4.8	-5.3
ugpE	glycerol-3-phosphate transporter subunit	-8.0	-7.8	-8.6
osmC	lipoyl-dependent Cys-based peroxidase, hydroperoxide resistance; salt-shock inducible membrane protein; peroxiredoxin	-9.3	-5.3	-5.2
srlD	sorbitol-6-phosphate dehydrogenase	-9.3	-7.1	-7.3
yahO	periplasmic protein, function unknown, YhcN family	-9.6	-5.2	-6.2
ugpB	glycerol-3-phosphate transporter subunit	-9.7	-11.5	-8.7
hdeB	acid-resistance protein	-10.3	-7.4	-7.1
srlE	glucitol/sorbitol-specific enzyme IIB component of PTS	-10.6	-8.3	-10.3
srlA	glucitol/sorbitol-specific enzyme IIC component of PTS	-15.1	-8.0	-10.9
cyoD	cytochrome o ubiquinol oxidase subunit IV	-19.5	-30.3	-26.2
cyoA	cytochrome o ubiquinol oxidase subunit II	-19.6	-25.4	-23.3
fliC	flagellar filament structural protein (flagellin)	-20.8	-5.1	-4.6
cyoE	protoheme IX farnesyltransferase	-21.1	-25.0	-27.1
cyoC	cytochrome o ubiquinol oxidase subunit III	-22.7	-31.1	-25.0
cyoB	cytochrome o ubiquinol oxidase subunit I	-22.8	-29.4	-24.7

The expression of YedWV increases the expression of several genes in the wildtype MG1655, Δ*cusRS*$_{MG}$ and Δ*cusR*$_{MG}$ strains. Differences in the fold change of *yedW* and *yedV* in the Δ*cusRS*$_{MG}$ and Δ*cusR*$_{MG}$ experiments, though they should be similar due to expression from a plasmid, can be observed. The induction of the genes in Δ*cusRS*$_{MG}$ is 7.4 and 3.8 times less than in the wildtype. In contrast to this the Δ*cusR*$_{MG}$ experiment exhibits fold changes values 23.5 and 53.9 times higher for *yedW* and *yedV* than in the wildtype.

YedWV expression leads to a conspicuous increase in the expression of genes like *hiuH*, *yedY* and *yedZ* which are upregulated more than 100-fold in the wildtype, other genes like *yghW*, *iraM*, *cusC* and *hybA* are regulated more than 20-fold. These genes show an interesting expression pattern as they are less upregulated in the Δ*cusRS*$_{MG}$ YedWV strain which is lacking the entire CusS/ CusR TCS. In Δ*cusR*$_{MG}$ YedWV *hiuH*, *yedY*, *yedZ*, *yghW* and *iraM* exhibit a higher fold change than in the MG1655 YedWV wildtype. The *hiuH* gene shows the highest positive fold change of 306.6-fold and an increase of 1994.4-fold in Δ*cusR*$_{MG}$. As no strong downregulation of the gene in the knockout strains can be seen (Table 3.3.2) this is almost completely due to the expression of YedWV which seems to be further amplified if the CusR RR is missing. This observation could be due to a role of the solitary CusS HK in the YedV/ YedW signaling. For instance CusS could increase the level of phosphorylated and active YedW RR either through phosphorylation of the YedV HK or by direct phosphorylation of the YedW RR.

Among the genes with fold changes more than 4 in the experiment MG1655 pVS198 → MG1655 YedWV many show similar regulation in the Δ*cusRS*$_{MG}$ pVS198 → Δ*cusRS*$_{MG}$ YedWV and Δ*cusR*$_{MG}$ pVS198→ Δ*cusR*$_{MG}$ YedWV experiments. These genes are for instance involved in electron transfer over the cytoplasmic membrane, in cell adhesion, in transport or are chaperones and members of the Mg^{2+} regulon. Also the TCS RstB/ RstA TCS is among the affected genes. Therefore one can assume that if they are directly regulated by the YedV/ YedW TCS they are independent from the CusS/ CusR TCS. Most downregulated genes and those with a positive fold change between -4 and -9 are similarily regulated in all three strains as well. The highest downregulation in the MG1655 YedWV experiment is -22.8-fold for the *cyoB* gene, others are involved in the respiratory chain, the PTS system or in general the uptake and metabolism of sugars.

The HK of the ArcB/ ArcA TCS which represses the respiratory metabolism is also slightly downregulated in strains overexpressing YedVW.
The genes affected by YedWV expression (Table 3.3.11) were used for a motif analysis to discover a shared promoter sequence used by the YedW RR. Our colleague Bhaswar Ghosh used the MEME motif discovery tool [217] to identify motifs in the 100 bp upstream region of the gene operons (Figure 3.3.12)

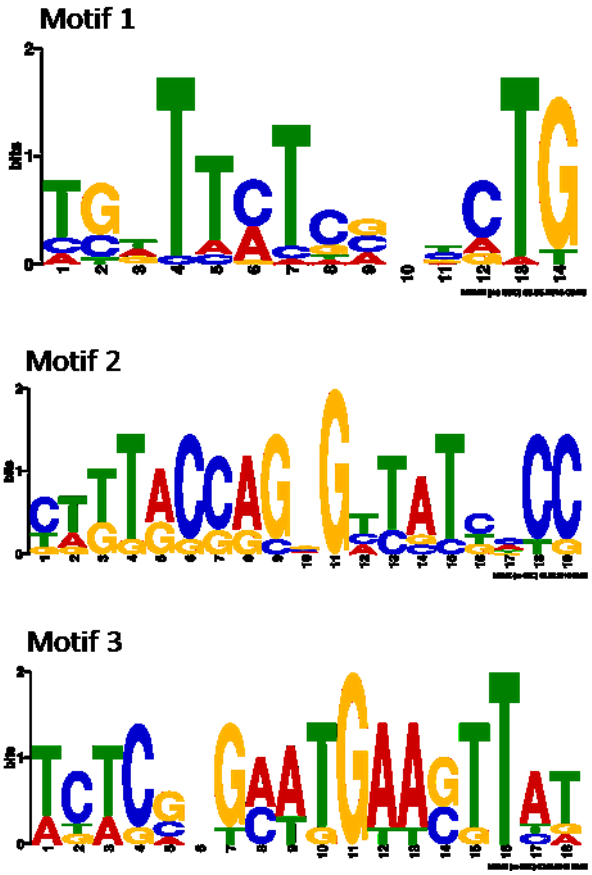

Figure 3.3.12 Potential binding motifs for YedW RR identified in the upstream regions of genes affected by YedWV expression.

Motif 1 (TXNTTNTCNNXCTG) is the most abundant motif present in almost all analysed genes. It is also present in the *yedW-hiuH* intergenic region as well as upstream of the *cusC* operon. The region upstream of *yedYZ* additionally also contains motif 3, which is also the sole motif upstream of cyoA. *FliC* only shows motif 2 in its

upstream region. Recently Urano and colleagues described a YedW consensus sequence based on DNase I footprinting analysis to be CATNACAANNTTGTAATG [53]. Also the CusR-binding sequence has been redefined from AAAATGACAANNTTGTCATTTT [29] to ATNACAANNTTGTAAT [53]. None of the motifs identified in the MEME analysis of genes regulated upon YedWV expression is conspicuously similar to the binding sites identified by DNAse I footprinting.

3.3.3.2 YedV expression

Upon overexpression of YedWV in the MG1655 wildtype a difference in the induction of the HK gene *yedV* and the RR gene *yedW* is observed even though they are expressed as one operon from the same plasmid. This indicates an operon intrinsic lower expression of the HK gene in the autoregulatory feedback loop of the TCS.

To investigate the effect of a higher HK copy number the impact of the YedV HK expression on the gene expression pattern in MG1655 wildtype, Δ*cusR*$_{MG}$ and Δ*cusRS*$_{MG}$ strains was studied (Scatter plots Figure 3.3.13).

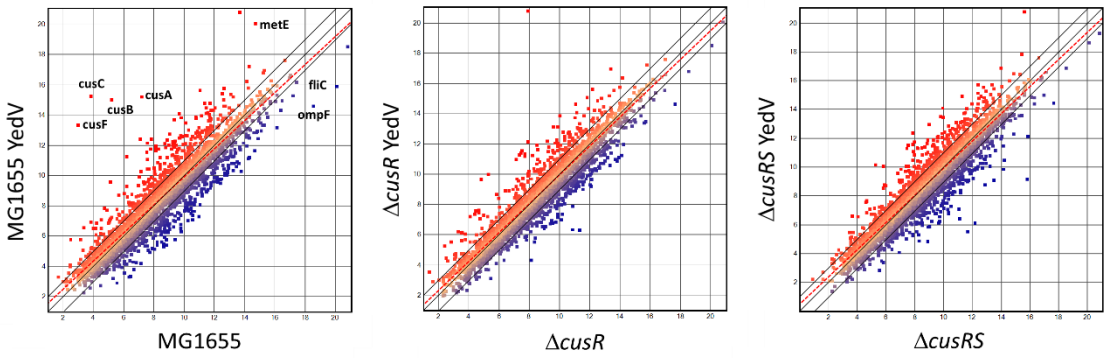

Figure 3.3.13 Scatter plots of YedV overexpression in MG1655, Δ*cusR*$_{MG}$ and Δ*cusRS*$_{MG}$

In the MG1655 wildtype the scatter plots reveal a positive effect of YedV on the induction of genes of the *cusCFBA* operon and on *metE* which is involved in the final step of the methionine biosynthesis [198]. The in general highly expressed *fliC* and *ompF* are among the genes which exhibit a negative fold change upon YedV expression. The regulation of these genes cannot be observed in the Δ*cusR*$_{MG}$ and Δ*cusRS*$_{MG}$ strains were the expression of YedV does not lead to an obvious change in gene expression.

The genes which are highly affected in the wildtype can be found in Table 7.1.9 in the Appendix. Fold change values of genes in strains expressing YedV from a plasmid are summarized in Table 3.3.12.

Table 3.3.12 Fold change more than 8 in YedV-overexpressing strains. Samples sorted by fold changes in MG1655.

Gene	Annotation	MG1655 pVS198 → MG1655 YedV	Δ*cusRS*$_{MG}$ pVS198 → Δ*cusRS*$_{MG}$ YedV	Δ*cusR*$_{MG}$ pVS198 → Δ*cusR*$_{MG}$ YedV
cusC	copper/silver efflux system, outer membrane component	2756.6	2.0	7.2
cusF	periplasmic copper- and silver-binding protein	1351.9	1.4	7.5
cusB	copper/silver efflux system, membrane fusion protein	915.8	1.6	8.1
cusA	copper/silver efflux system, membrane component	265.4	1.3	3.0
yedV	putative sensory kinase in two-component regulatory system with YedW	142.8	37.6	7698.7
metE	5-methyltetrahydropteroyltriglutamate- homocysteine S-methyltransferase	41.5	-2.7	-3.0
sbp	sulfate transporter subunit	40.4	16.0	6.0
ibpB	heat shock chaperone	34.0	19.5	26.6
ibpA	heat shock chaperone	24.5	13.4	16.1
mmuP	CP4-6 prophage, predicted S-methylmethionine transporter	24.2	-1.6	-6.4
metL	fused aspartokinase II/homoserine dehydrogenase II	24.2	-1.3	-4.5
yncJ		22.5	29.6	29.0
metF	5,10-methylenetetrahydrofolate reductase	21.3	-1.7	-7.0
metB	cystathionine gamma-synthase, PLP-dependent	20.8	-2.1	-7.6
tqsA	pheromone AI-2 transporter	16.9	16.2	15.4
cpxP	inhibitor of the cpx response, periplasmic adaptor protein	16.7	18.8	14.6
yebE	inner membrane protein, DUF533 family	15.7	23.5	18.7
ybdL	methionine aminotransferase, PLP-dependent	14.1	-2.2	-7.1
yneM	expressed protein, membrane-associated	13.5	13.6	9.8
ycfS	L,D-transpeptidase linking Lpp to murein	13.1	13.9	16.6
fxsA	suppressor of F exclusion of phage T7	12.1	9.2	12.9
spy	periplasmic ATP-independent protein refolding chaperone, stress-induced	11.7	16.2	11.7
ydeH	diguanylate cyclase, required for pgaD induction	11.4	14.6	14.7
ycjX	conserved protein with nucleoside triphosphate hydrolase domain	11.4	8.2	10.8
acrD	aminoglycoside/multidrug efflux system	11.1	9.2	8.2
htpX	putative endopeptidase	10.7	12.4	12.1
pheP	phenylalanine transporter	10.6	1.0	1.3
chaA	calcium/sodium:proton antiporter	10.6	11.2	12.1
iraM	RpoS stabilizer during Mg starvation, anti-RssB factor	9.7	3.5	6.5
ydeS	putative fimbrial-like adhesin protein	9.3	7.9	4.8
clpB	protein disaggregation chaperone	8.9	10.2	11.2

Gene	Annotation	MG1655 pVS198 → MG1655 YedV	Δ*cusRS*$_{MG}$ pVS198 → Δ*cusRS*$_{MG}$ YedV	Δ*cusR*$_{MG}$ pVS198 → Δ*cusR*$_{MG}$ YedV
ydeR	putative fimbrial-like adhesin protein	8.9	5.6	6.1
cysD	sulfate adenylyltransferase, subunit 2	8.9	4.0	1.3
rrsD	16S ribosomal RNA of rrnD operon	8.8	3.3	2.5
ybdM		8.7	-2.5	-5.7
bhsA	biofilm, cell surface and signaling protein	8.6	4.9	4.0
cysN	sulfate adenylyltransferase, subunit 1	8.6	3.9	1.4
metA	homoserine O-transsuccinylase	8.5	-1.2	-5.1
ycjF	inner membrane protein, UPF0283 family	8.5	7.2	8.7
cysP	thiosulfate-binding protein	8.5	3.6	1.3
pspD	peripheral inner membrane phage-shock protein	8.2	6.6	5.5
cysU	sulfate/thiosulfate ABC transporter permease	8.1	3.3	1.5
yciW	putative oxidoreductase	8.1	3.7	1.4
flgE	flagellar hook protein	-8.0	-6.2	-4.6
katE	catalase HPII, heme d-containing	-8.1	-3.7	-3.3
intG		-8.3	-5.4	-3.0
glpQ	periplasmic glycerophosphodiester phosphodiesterase	-8.4	-13.0	-6.8
ycaC	putative hydrolase, isochorismatase family	-8.5	-4.4	-6.0
yciF	putative rubrerythrin/ferritin-like metal-binding protein	-8.6	-4.3	-3.9
glpT	sn-glycerol-3-phosphate transporter	-8.6	-19.8	-6.4
ydcT	putative spermidine/putrescine transporter subunit	-8.8	-8.3	-5.5
lamB	maltose outer membrane porin (maltoporin)	-9.0	-11.1	-7.0
poxB	pyruvate dehydrogenase (pyruvate oxidase), thiamin-dependent, FAD-binding	-9.1	-3.9	-3.8
ydcS	polyhydroxybutyrate (PHB) synthase, ABC transporter periplasmic binding protein homolog	-9.2	-6.0	-4.3
malK	fused maltose transport subunit, ATP-binding component of ABC superfamily/regulatory protein	-10.0	-16.2	-6.0
yahO	periplasmic protein, function unknown, YhcN family	-10.5	-2.1	-3.2

Genes the highest affected by the expression of YedV in the wildtype belong to the CusCFBA copper-efflux transporter. Their upregulation is dependent on the presence of the CusR RR. Also dependent on the CusR RR is the upregulation of *metE*, *sbp*, *mmuP*, *metL*, *metF* and *metB* genes. Upregulation by YedV expression in all three MG1655, Δ*cusRS*$_{MG}$ and Δ*cusR*$_{MG}$ strains can be observed for instance for the heat shock chaperones IbpB and IbpA, the pheromone auto-inducer-2 transporter TqsA or the inhibitor of the CpxA/ CpxR response CpxP along with other genes involved in transport and cell adhesion. Most of the downregulated genes in MG1655 YedV are similarly regulated in the other two strains.

Genes like *hiuH*, *yedZ*, *yedY* and *yedW* which show high fold changes in the YedVW overexpression experiment are not affected by the overexpression of YedV in the wildtype.

3.3.3.3 YedW expression

Genes activated by YedW overexpression can implicate regulatory targets of the RR [54], [210]. I looked at the gene expression pattern in Δ*cusR*$_{MG}$ and ΔcusRS$_{MG}$ expressing YedW from a plasmid (scatter plot Figure 3.3.14) and their fold changes (Table 3.3.13).

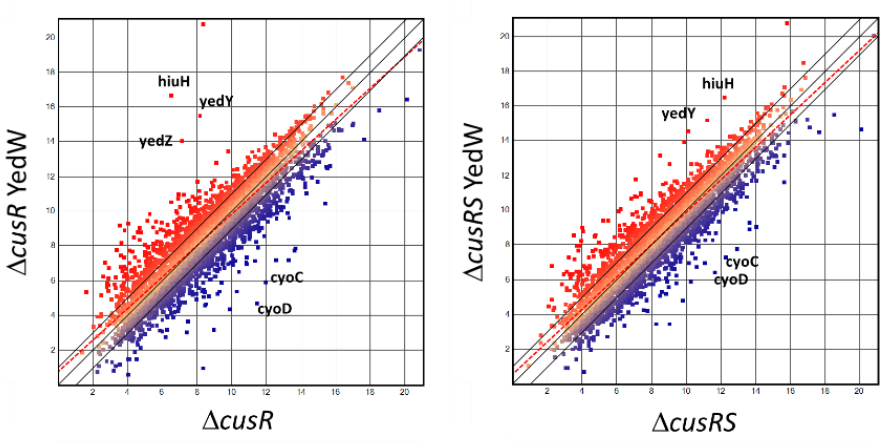

Figure 3.3.14 Scatter plots of single YedW overexpression in Δ*cusR*$_{MG}$ and Δ*cusRS*$_{MG}$

The scatter plots of Δ*cusR*$_{MG}$ YedW and Δ*cusRS*$_{MG}$ YedW both show conspicuous upregulation of *hiuH*, *yedY* and *yedZ* as well as downregulation of *metE*, *fliC* and *cyo* genes indicating similar gene regulation in both experiments. I looked at the fold changes in knockout strains expressing YedW. A fold change more than 8 in Δ*cusRS*$_{MG}$ pVS198 → Δ*cusRS*$_{MG}$ YedW was used to select genes.

Table 3.3.13 Fold change more than 8 in YedW-overexpressing strains.
Samples sorted by fold changes in Δ*cusRS*$_{MG}$.

Gene	Annotation	Δ*cusRS*$_{MG}$ pVS198 → Δ*cusRS*$_{MG}$ YedW	Δ*cusR*$_{MG}$ pVS198 → Δ*cusR*$_{MG}$ YedW
yjbI		33.5	33.7
yedW	putative DNA-binding response regulator in two-component system with YedV	32.6	5654.6
cusC	copper/silver efflux system, outer membrane component	31.9	21.3
ygeH	Predicted transcriptional regulator	27.1	49.8
uidC	putative outer membrane porin protein	26.9	8.4
uidB	glucuronide transporter	26.1	13.1
wza	lipoprotein required for capsular polysaccharide translocation through the outer membrane	23.2	20.5
uidA	beta-D-glucuronidase	22.0	13.2
hiuH	hydroxyisourate hydrolase	20.6	1182.6
ygaQ		20.0	17.8
yafT	lipoprotein	20.0	22.7
yjfZ		18.4	20.4
yeaI	putative membrane-anchored diguanylate cyclase	17.9	23.4
yhaI	inner membrane protein, DUF805 family	16.9	10.9
yedZ	inner membrane heme subunit for periplasmic YedYZ reductase	16.5	124.2
yedY	membrane-anchored, periplasmic TMAO, DMSO reductase	16.5	164.7
yghW		15.3	39.6
bglG	transcriptional antiterminator of the bgl operon	15.3	21.0
hybB	putative hydrogenase 2 cytochrome b type component	15.0	13.9
hybA	hydrogenase 2 4Fe-4S ferredoxin-type component	14.7	24.2
hybO	hydrogenase 2, small subunit	14.6	16.4
yhcA	putative periplasmic chaperone protein	14.0	19.3
ycgH		13.9	16.7
yfdE	putative CoA-transferase, NAD(P)-binding	13.7	16.1
rzpQ	Rz-like protein, Qin prophage	12.8	12.0
glcC	DNA-binding transcriptional dual regulator, glycolate-binding	12.8	9.3
mhpR	DNA-binding transcriptional activator, 3HPP-binding	12.6	9.2
recN	recombination and repair protein	12.5	13.0
yejG		11.8	13.4
yjiC		10.7	5.8
gfcB	putative outer membrane lipoprotein	10.5	14.1
yjcS	conserved protein, metallo-beta-lactamase superfamily	9.2	13.2
ydeT		8.6	12.7
ydjE	putative transporter	8.4	10.7
pinE	e14 prophage, site-specific DNA recombinase	8.4	5.7
fliC	flagellar filament structural protein (flagellin)	-8.1	-6.4
ugpA	glycerol-3-phosphate transporter subunit	-8.3	-10.3
ompF	outer membrane porin 1a (Ia, b, F)	-8.7	-10.9
yeaD		-8.8	-7.0
pgsA	phosphatidylglycerophosphate synthetase	-9.0	-11.8

Gene	Annotation	Δ*cusRS*$_{MG}$ pVS198 → Δ*cusRS*$_{MG}$ YedW	Δ*cusR*$_{MG}$ pVS198 → Δ*cusR*$_{MG}$ YedW
osmC	lipoyl-dependent Cys-based peroxidase, hydroperoxide resistance; salt-shock inducible membrane protein; peroxiredoxin	-9.4	-9.5
sra	stationary-phase-induced ribosome-associated protein	-9.4	-13.5
iraP	anti-RssB factor, RpoS stabilizer during Pi starvation; anti-adapter protein	-9.6	-13.7
gabP	gamma-aminobutyrate transporter	-9.7	-14.9
csiD	carbon starvation protein	-10.4	-11.4
ugpB	glycerol-3-phosphate transporter subunit	-10.4	-7.4
yahO	periplasmic protein, function unknown, YhcN family	-12.0	-14.4
ykgO	RpmJ-like protein	-12.4	-14.9
ykgM	50S ribosomal protein L31 type B, alternative L31 utilized during zinc limitation	-14.5	-12.1
gapA	glyceraldehyde-3-phosphate dehydrogenase A	-15.7	-30.6
malE	maltose transporter subunit	-16.8	-11.5
yncE	ATP-binding protein, periplasmic, function unknown	-19.9	-65.5
srlD	sorbitol-6-phosphate dehydrogenase	-20.4	-29.9
cyoE	protoheme IX farnesyltransferase	-25.2	-59.7
cyoC	cytochrome o ubiquinol oxidase subunit III	-30.7	-65.0
cyoB	cytochrome o ubiquinol oxidase subunit I	-30.9	-54.7
srlE	glucitol/sorbitol-specific enzyme IIB component of PTS	-31.7	-45.7
cyoA	cytochrome o ubiquinol oxidase subunit II	-34.1	-40.0
cyoD	cytochrome o ubiquinol oxidase subunit IV	-37.0	-108.4
metE	5-methyltetrahydropteroyltriglutamate- homocysteine S-methyltransferase	-43.0	-12.1
srlA	glucitol/sorbitol-specific enzyme IIC component of PTS	-47.3	-54.5

The expression of YedW induces genes like for instance *yjbI*, *cusC*, *ygeH*, *uidC* up to over 30-fold in Δ*cusRS*$_{MG}$ pVS198 → Δ*cusRS*$_{MG}$ YedW. Among the upregulated genes are also *hiuH*, *yedZ* and *yedY* which show a strikingly higher upregulation in Δ*cusR*$_{MG}$ YedW. Likewise *yncE* and several *cyo* genes are more downregulated in *cusR*$_{MG}$ YedW than in Δ*cusRS*$_{MG}$ YedW. Neither of the genes highly regulated in Δ*cusR*$_{MG}$ upon YedW expression show clear differences in regulation in Δ*cusR*$_{MG}$ pVS198 or Δ*cusRS*$_{MG}$ pVS198 in comparison with the wildtype. One possible explanation for this observation could be an increased kinase activity of CusS on the YedW RR due to the lack of the cognate RR. In this case YedW is expressed from a plasmid and therefore also shows high upregulation in Δ*cusR*$_{MG}$ but is originally it is downregulated in Δ*cusR*$_{MG}$ pVS198 (Table 3.3.2).

Most genes are similarly up- or downregulated in Δ*cusR*$_{MG}$ YedW and Δ*cusRS*$_{MG}$ YedW. Other upregulated genes which are regulated similarily in both knockout strains are members of the *hybOABCDEFG* operon and are involved in the respiratory chain.

4 Discussion

The enterobacterium *E. coli* is a versatile organism which occurs in very different habitats where it is faced with a variety of environmental challenges. One crucial feature therefore is to adjust its metabolism and lifestyle accordingly. An important part of the detection of external stimuli is played by TCSs, which then alter the expression of target genes. Bacterial genomes often contain a multitude of paralogous TCSs genes. As each of the HKs detects different environmental stimuli, TCS signaling can occur in parallel and the information is integrated into an overall cell response. The conserved TCS structure of HK and RR, which share a certain homology with other TCSs, can result in signal integration among different systems. Cross-talk is a favourable interaction between different non-cognate TCSs and can occur between HKs, between HK and RR and between different RRs. The interaction can result in changes of the phosphorylation state of the non-cognate components which influences downstream signaling. The objective of this work was the identification of interaction between the components and how this interaction influences the signaling within and among TCSs. We wanted to understand how the bacterium generates an overall cell response to a variety of simultaneously detected stimuli. These insights can for instance put to use to inhibit virulence conferring TCSs in pathogenic bacteria.
Identified protein interactions led to the more detailed investigation of the TCS CusS/ CusR, YedV/ YedW and to a smaller proportion BaeS/ BaeR. A possible regulatory interaction between the TCSs was assumed and the question of signal integration between the systems was addressed by studying the TCSs' effect on specific and global gene expression.

4.1 Protein interactions and cross-talk

Interactions among the TCS's components are a prerequisite for phosphoryl-group transfer, signaling and cross-talk. Studying *in vivo* interactions of the *E. coli* TCSs using acceptor photobleaching FRET measurements is a means to search for interactions relevant to bacterial signaling. In our lab this method was used in previous studies to

research homo-oligomerization of cognate HKs and identified positive interactions for 65 % of the 17 tested HKs (Table 3.1.3). Among them are for instance YedV and BaeS [203]. The study also found interactions within the TCS BaeS/ BaeR, BasS/ BasR, CpxA/ CpxR, CusS/ CusR, EnvZ/ OmpR, PhoQ/ PhoP, QseC/ QseB and YedV/ YedW [203]. We additionally identified interactions among the cognate HK-RR pairs of the BarA/ UvrY, CitA/ CitB, DcuS/ DcuR, NarX/ NarL, TorS/ TorR, UhpB/ UhpA, YpdA/ YpdB TCSs.

Acceptor photobleaching FRET experiments identify interactions of proteins when their intermolecular distance is below 10 nm. Most of the tested cognate TCS proteins show positive interactions within the TCS, an evidence that our method identifies relevant component interactions. Interactions between the HK and RR as well as the dimerization of HKs take place at the DHp domain which is located between the last TMH and the C-terminal HATPase domain. The fluorophore is C-terminally fused to the HATPase domain in the cytoplasm. It could block the RR's access to the interaction site. This can be the reason why we fail to identify positive interactions for all cognate pairs. An additional factor can be that components might require the presence of a certain stimulus to trigger interaction. As we performed the steady-state experiments without stimuli we might fail to identify interactions which only happen upon stimulation.

Relevant for the identification of cross-talk between different TCSs are interactions between non-cognate HKs and RRs. An *in vitro* screen performed by Yamamoto and colleagues identified 22 phosphorylation events among non-cognate HK-RR pairs, i.a. also phosphorylation of the CusR RR by the HKs YedV and BaeS [103]. A previous *in vivo* study in our lab identified interactions between non-cognate HKs as well as between non-cognate HKs and RRs [203]. Tested non-cognate HK-RR pairs only revealed 6 out of 38 pairs to show positive interactions. It is therefore likely that the observed interactions are also specific interactions. Based on findings of our collaborator, who found homo-oligomerization of the HKs CusS, YedV and BaeS upon copper stimulation, steady-state interactions between non-cognate components were identified. Positive interactions were found i.a. between the YedV and BaeS HKs as well as between the HK-RR pairs CusS-YedW and YedV-CusR (Table 3.1.4). We focused on investigating interconnection and cross-talk among the three TCSs

CusS/ CusR, YedV/ YedW and BaeS/ BaeR. The identified interactions within and among these systems are depicted in Figure 4.1.

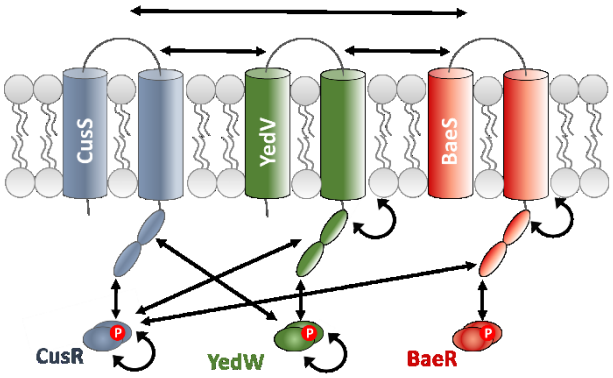

Figure 4.1 Interaction model of the CusS/ CusR, YedV/ YedW and BaeS/ BaeR TCSs based on observed steady-state FRET interactions.

We observed that the three systems interact on the HK level, giving rise to potential hetero-oligomerization or formation of higher-order complexes which could in turn impact downstream signaling by integrating the response of different HKs into coordinated phosphorylation events. One cannot distinguish between the formation of dimers and the formation higher-order complexes due to non-sufficient spatial resolution in the experimental setup. We were primarily interested whether the cross-interaction of components results in a physiological relevant cross-talk. Therefore the composition of potential higher order signaling complexes was of minor interest for the time being. A HK-pull-down experiment could not provide additional confirmation of HK interactions under unstimulated conditions. In general the protein copy number of HKs is low and often gets amplified only upon induction of the autofeedback loop of the TCS. An experiment performed under TCSs stimulating condition could therefore have identified more proteins due to the increased copy number of potentially interacting HKs.

Non-cognate HK-RR interaction experiments of the CusS/ CusR, YedV/ YedW and BaeS/ BaeR TCS show interaction of CusS with YedW as well as interaction of BaeS with the CusR RR. An interaction of YedV with CusR was reported before (Table 3.1.4) and also seen in the *in vitro* phosphorylation experiments [103], but was not supported

by my experiment. These experiments showed a high standard error due to a high variation between the results of independent measurements and therefore might actually fail to identify positive interaction like seen before.

4.2 Effects of interactions on gene expression

4.2.1 Effect on expression of promoter-GFP reporter

Target gene expression can help describing the impact of component interactions on the TCS signal-transduction. To elucidate whether the interactions observed between the CusS/ CusR and the YedV/ YedW TCSs affect gene expression we studied the expression profiles of target genes using promoter-GFP fusions of the *cusC*, *yedW* and *hiuH* (*yedX*) genes (Figure 2.3.1). The *cusC* gene encodes for a copper-efflux transporter, *yedW* and *hiuH* genes share the same intergenic region and encode for the YedW RR and a 5-hydroxyisourate hydrolase. The expression of the *cusC* gene is known to be regulated in a CusR dependent manner whereas a regulation of the *yedWV* operon was assumed based on the finding that the transcriptional activation of *yedV* is also CusR-dependent [29]. As many TCSs possess an autoregulatory feedback loop [29], [42], [218]–[220] a regulation of *yedW* by the YedV/ YedW TCS is likely. In fact the upstream regions of the three genes were recently described to be bound by both the CusR and YedW RR [53]. In our experiments the promoter-GFP fusions of *cusC* and *hiuH* exhibit a low basal expression. Contrary to that the *yedW* gene's basal expression with 793 RFUs is significantly higher. Copper, a described stimulus of the CusS/ CusR TCS [28], can induce the expression of *cusC* 196 times whereas the expression of *yedW* and *hiuH* is only induced 6 and 8 times. As *cusC* is tightly regulated under non-induced conditions and highly upregulated upon activation of the TCS signaling with $CuSO_4$ it was selected for further experiments investigating cross-talk.

The lack of CusS or CusR components causes the increase in expression of *cusC* and *yedW* triggered by copper in the wildtype to be only 2-times or none at all (Figure 3.2.2). Copper sensing by the CusS HK is crucial for the high transcriptional activation of the *cusC* promoter reporter. The CusS protein carrying mutations in potential copper binding sites fails to activate gene transcription significantly (Figure 3.2.3). Though the activation of *cusC* is primarily CusR-dependent we find that the lack of the YedV HK

or the lack of the YedW RR diminishes the overall activation of the promoters below the activation in the wildtype (Figure 3.2.2). This decrease is evidence for an intertwined signaling between the two TCSs. Only the presence of both the CusS/ CusR and the YedV/ YedW TCSs results in the activation observed upon $CuSO_4$ stimulation in the wildtype. The CusS/ CusR is essential for the high activation, whereas YedV/ YedW contributes a small proportion to the overall activation. The strain termed Δ*cusRS*$_{MG}$ was derived from a Keio collection strain [194] which was described to be deficient for the *cusS* gene. However the way the Keio collection strain was created damaged the stop codon of the upstream CusR RR as well. Therefore the correct transcription of *cusR* is impaired and the strain was renamed to Δ*cusRS*$_{MG}$. Experiments with the false strain led to the later rejected assumption that YedV might play a role in the phosphorylation of the CusS HK, as YedV overexpression in this strain did not affect *cusC* like observed in the wildtype. In the wildtype a fully functional YedV is required for an activating effect on *cusC* transcription (Figure 3.2.4). The expression of YedV, YedV$_{H245Q}$ and YedV with a mutated HATPase (CA) domain in MG1655 background showed that only YedV can induce *cusC* expression in the absence of a copper stimulus. YedV overexpression mimicking HK activation in the Δ*cusS*$_{251\text{-}1443}$ strain, which is deficient for only the HK, revealed that YedV influences the CusS/ CusR signaling by HK-RR cross-talk (Figure 3.2.5). The experiments with YedV mutations showed that a functional HATPase (CA) domain of YedV is required for the observed cross-talk reaction. The mutation of the conserved histidine residue on the other hand resulted in a promoter expression similar to the one of YedV. This could be due to the formation of dimers between functional YedV and YedV$_{H245Q}$ and a subsequent phosphorylation of the conserved His residue of YedV in *trans*.

We also observe a slight upregulation of *cusC* in the Δ*cusS*$_{251\text{-}1443}$ with overexpressed YedV upon copper stimulation though the characterized copper-sensing HK CusS is missing raising the question whether YedV can sense $CuSO_4$. The periplasmic domains of CusS and YedV distinctly differ in size by about 20 amino acids and they do not share the copper-binding residues annotated for CusS. YedV might bind copper in different way or it might be activated by an unknown input stimulus. This stimulus is likely related to copper stress and should activate *cusC* and *yedW* promoter-GFP reporter expression. In this screen several oxidative stress inducing chemicals as well as

metals were tested non-conclusively. Also H_2O_2,which has been reported to increase the amount of transcribed *hiuH* RNA in a YedV/ YedW dependent manner [53], did not have an effect on the promoter-GFP transcriptional reporters in our experiments. As we measured the amount of GFP expression after translation we cannot exclude that H_2O_2 has an effect on the gene transcription, but have to speculate about whether the upregulation of transcription is not high enough to result in a detectable increase in the protein copy number of HiuH.

Our data suggest that the two TCS CusS/ CusR and YedV/ YedW are cooperating in the activation of target gene expression. The copper induced activation of *cusC* and *yedW* expression is primarily dependent on the presence of the CusS/ CusR TCS but the YedV/ YedW system also contributes to the overall activation of gene expression by cross-talk between the YedV HK and the CusR RR as well as possibly by a small amount of direct promoter regulation through signaling within the YedV/ YedW TCS. Whether this is due to the activation of signaling by the recognition of a stimulus or whether it happens even in the absence of a stimulus cannot be inferred from this data.

4.2.2 Effect on transcriptomic data

RNA isolation and sequencing was performed with the objective to broaden the knowledge on the YedV/ YedW, CusS/ CusR and BaeS/ BaeR regulons. Based on the observed impact of $CuSO_4$ on homo-oligomerization of the CusS, YedV and BaeS HKs we studied overall gene transcription in RR deficient strains in a copper-dependent manner. Changes in gene transcription would indicate an involvement of the TCS in the copper regulon. We further investigated whether the in the promoter activation assays observed cooperation between YedV/ YedW and CusS/ CusR influences the overall gene transcription. This was studied in several deletion strains expressing components of the TCSs.

In the wildtype BW25113 $CuSO_4$ induces the expression of genes which are described to be involved in the copper homeostasis like the *copA* and the *cusCFBA* operon [221], [176]. A previous study on the effect of copper on gene expression by Kershaw *et al.* reported an effect on stress response genes triggered by CpxR/ CpxA TCS signaling induced by toxic effects of copper on the cell. They speculated about a copper-induced downregulation of genes like *cyoBC*, encoding for proteins of the respiratory chain,

which they find to be involved in the iron uptake regulon [221]. Our wildtype also shows a copper-dependent upregulation of the *cpxP* and *spy* genes which are controlled by the CpxR/ CpxA TCS. A downregulation of *cyo* genes was observed in several tested strains but not in BW25113. We additionally found genes involved in the zinc homeostasis as well as *hiuH* to be upregulated in the BW25113 upon stimulation with copper.

The CusS/ CusR TCS was researched with RNA sequencing of several strains (Δ*cusR*$_{BW}$, Δ*cusR*$_{BW}$ + 1 mM $CuSO_4$, Δ*cusRS*$_{MG}$, Δ*cusR*$_{MG}$ and MG1655 CusR). The RNA sequencing identified the CusR RR to be crucial for a copper mediated activation of the transcription of *cusCFBA* and *hiuH* genes. This was recently also described by Urano and colleagues [53]. Less strongly affected are the zinc transporter subunit ZnuA and the inhibitor of the Cpx response CpxP. The ZinT binding protein is partially affected by the lack of CusR (Table 3.3.1). Interestingly the *zinT* gene is located downstream of *hiuH* and the *yedYZ* operon. This could hint at a potential connection of the expression of these genes. We did not observe an effect of zinc on the promoter-GFP reporter expression in our screen for activating stimuli. This indicates that zinc does not stimulate signaling, but as zinc and copper share a similar molecular weight a potential involvement of the CusS/ CusR TCS in the regulation of the zinc transporter might contribute to the export of copper from the cell.

In the MG1655 background the effect of the lack of CusR and of the entire CusS/ CusR TCS without copper stimulus was assessed. In both strains the expression of the *yedW*, *yedV* and *hiuH* genes differ from the wildtype however the lack of the entire *cusRS* operon has a slight positive effect on their expression compared to a strong negative effect observed in Δ*cusR*$_{MG}$ (Tables 3.3.2, 3.3.3 and 3.3.4). The upregulation of *yedWV* and *hiuH* in the absence of the entire CusS/ CusR TCSs indicate a possible involvement of the YedV/ YedW TCS in their expression. A strong downregulation of the *yedWV* and *hiuH* genes in Δ*cusR*$_{MG}$ compared to Δ*cusRS*$_{MG}$ suggests that CusS might exhibit a phosphatase activity onto low copy numbers of YedW, interfering with its positive influence on gene expression.

The comparison of the MG1655 wildtype with a strain overexpressing CusR confirms the induction of the *cusC* operon whereas the *hiuH* gene exhibits a downregulation by the CusR RR (Table 3.3.5 and 3.3.6). The downregulation of *hiuH* is contradictory to

the finding of the copper-stimulation experiment, where CusR is required for a positive regulation of *hiuH*. On the other hand the *cusC* operon, which is also positively regulated in a CusR-dependent manner upon copper stimulation, is not down- but upregulated in this sample. Therefore an excess of CusR might have an inhibitory effect on some genes which are normally positively regulated. Possible is also a phosphorylation-dependent binding of CusR to promoter regions. As in this experiment the CusS signaling is not induced, CusR is not phosphorylated and might fail to bind certain promoter regions.
We also observe several other gene groups to be affected in our experiments with CusS/ CusR deficient strains. Several *arg* genes, which are involved in the arginine biosynthesis, are upregulated in the Δ*cusR*$_{BW}$ strain treated with copper. A similar effect has been previously described for *B. subtilis* when lacking of a global regulator of copper homeostasis [178]. We find this to also be the case in *E. coli* BW25113. *Met* genes, which are involved in the methionine biosynthesis, are upregulated if the CusS/ CusR TCS or parts of it are missing. The *met* genes have been shown to be negatively affected by oxidative stress [214]. The degree of oxidative stress might be different between the tested cultures. Also possible is, as copper is thought to be involved in the generation of reactive oxygen species, that the lack of CusS/ CusR signaling might revoke a negative impact on *met* gene expression. *Cyo* genes which are described to be downregulated in a copper-dependent manner [221] are also be negatively affect by the overexpression of the CusR RR in the MG1655 wildtype. This indicates an involvement of the CusS/ CusR TCS in the regulation of the *cyo* genes. So far the binding of CusR to the upstream region of *cyoA* has been inferred only based upon binding sequence similarities [53]. Whether the downregulation happens through direct negative regulation by CusR or as a secondary effect remains to be determined in more detail.

As self-interaction upon copper stimulation of the BaeS HK was observed, transcriptomic data was obtained for the Δ*baeR*$_{BW}$ strain with and without copper stimulation (Table 3.3.7 and 3.3.8). We compared our Δ*baeR*$_{BW}$ sequencing results to the ones obtained for Δ*cusR*$_{BW}$ and therefore observe a copper dependent downregulation of *arg* and *cyo* genes similar to the wildtype and an upregulation of the

cusC operon and the *hiuH* gene. No exclusive BaeS/ BaeR-mediated effect of copper on gene expression was observed. This weakens the assumption that BaeS might be directly involved in signaling in response to copper.

To shed light on the function and potential cross-talk of the YedV/ YedW TCS we performed several RNA sequencing experiments (Δ*yedW*$_{BW}$ with and without copper stimulus as well as YedV, YedW and YedWV expression in MG1655 wildtype, Δ*cusRS*$_{MG}$ and Δ*cusR*$_{MG}$). The RNA sequencing of Δ*yedW*$_{BW}$ stimulated with copper revealed that a lack of the YedW RR does not have an effect on gene expression in minimal A medium. Stimulation with copper does not alter the gene expression distinctively from the wildtype and all genes, including members of the copper regulon, are similarly expressed (Table 3.3.10).
The overexpression of YedV/ YedW components on the other hand revealed possible regulatory targets. The overexpression of YedWV resulted in an upregulation of *hiuH* and its downstream genes *yedY* and *yedZ* in the MG1655 wildtype background. Shortly before the end of this study the proteins of the *yedYZ* (*msrPQ*) operon were described to work as methionine sulfoxide reductase which protects proteins in the cell envelope from oxidative damage. The study also described an YedW dependent activation of *yedY* (*msrP*) transcription by hypochlorous acid [222]. These genes are less strongly induced by YedWV in Δ*cusRS*$_{MG}$ but much higher induced in strains lacking only the CusR RR (Table 3.3.11). This indicates an involvement of the CusS HK in the activation of the YedV/ YedW TCS by cross-phosphorylation of either the HK or the RR. In the knockout strains overexpressing only the RR YedW, we observe a similar regulatory pattern on *hiuH*, *yedY* and *yedZ* supporting a phosphorylation of YedW by CusS (Table 3.3.13). Cross-phosphorylation of the YedW RR is also supported by the finding of a recent study which showed that YedW-dependent activation of *yedY* (*msrP*) is further increased in a strain lacking the YedV HK than in the wildtype [222]. The *cusC* gene upon YedWV overexpression on the other hand does not show any differences in the level of upregulation between the wildtype and Δ*cusRS*$_{MG}$ but is less high upregulated in Δ*cusR*$_{MG}$. We assume a kinase activity of CusS on the YedW RR based on the gene transcription pattern of *hiuH*, *yedY* and *yedZ*. Our finding for *cusC* could imply a phosphatase activity of CusS counteracting the RR's phosphorylation by

its cognate HK YedV. Since kinase and phosphatase activity of a HK influences the overall level of phosphorylated RR in the cell a phosphatase activity would affect other genes in the same way. Therefore a phosphatase activity of the CusS HK is not likely to be the reason for the observed pattern. Rather the binding of the phosphorylated YedW RR to promoter regions might play a role. Possibly phosphorylated YedW does not bind the promoter region of *cusC* as strongly, hence a lower transcription. In support of different binding affinities is the overexpression of only YedW which did result in the induction of *cusC* expression in both strains, though it was again lower in $\Delta cusR_{MG}$. The high abundance of the RR YedW seems to induce expression of target genes in a differential manner dependent on the presence of the CusS HK.

When looking at gene groups affected by the overexpression of YedWV and YedW we observe a downregulation of *cyo* genes, like in the MG1655 CusR sample and like it was described for copper stress. This supports the indications that the two signaling pathways of YedV/ YedW and CusS/ CusR are interconnected. A SELEX screening identified a YedW binding site upstream of *cyoA* [53] but our search for a shared binding motif (described below) showed that *cyoA* does not share the most common motif found for genes strongly regulated upon YedV/ YedW overexpression. Therefore, like for the CusR RR, it remains to be determined whether the downregulation actually happens through direct negative regulation by the RR at the gene promoter.

We searched for a consensus RR binding sequence of YedW using the genes highly affected by YedWV overexpression (Table 3.3.11). We identified a common shared motif (TXNTTNTCNNXCTG) in the upstream regions. It is for instance present in the upstream regions of *yedWV*, *yedYZ, hiuH* and *cusC*. The promoter region of *yedYZ* contains in addition to the common shared motif another binding motif. This motif is also present in the upstream region of the *cyoA* operon. This might suggest that both operons are additionally or uniquely controlled by another unknown regulator.

YedV overexpression experiments were performed to mimic the activated YedV HK. They show a CusR-dependent effect on the *cusC* operon, as a high upregulation is only observed in the MG1655 but not in the knockout strains lacking the CusR RR (Table 3.3.12). A similar regulatory pattern can be found for the *met* genes. Compared to the samples overexpressing YedWV or YedW, YedV's effect on the *hiuH*, *yedY* and *yedZ* genes is low in the wildtype. This could be due to an only minimal amount of linear

signaling within the YedV/ YedW TCS due to low abundance of the RR YedW. Another explanation could be that if YedV phosphorylates CusR, the RR could bind the gene promoters with lower affinity than observed for the *cusC* gene.

Our RNA data suggests that the signaling of the phylogenetically close TCSs YedV/ YedW and CusS/ CusR [110] is interconnected. The data indicate that, as recently described the YedW and the CusR RRs are involved in the regulation of several shared target genes like *cusC* and *hiuH* [53]. Urano *et al.* suggested a repressor function of YedW on the transcription copper-containing cytochrome b_0 ubiquinole oxidase (*cyo* operon) of the respiratory chain [53]. Our findings furthermore show the same role for the CusR RR, as both YedW and CusR overexpression result in a downregulation of these genes. Our data suggests that high levels of CusR favours the transcription of the *cusC* operon over other target genes. The same effect is seen for YedW, which mainly activates *hiuH*, *yedY* and *yedZ* and only to a lesser extent *cusC*. A possible explanation of our observations could be differences in binding affinities of the two RRs to the promoter regions of the target genes. A model summarizing the signaling of the CusS/ CusR and YedV/ YedW TCSs based on our transcriptomic data and the promoter activation assays can be found in Figure 4.2 below.

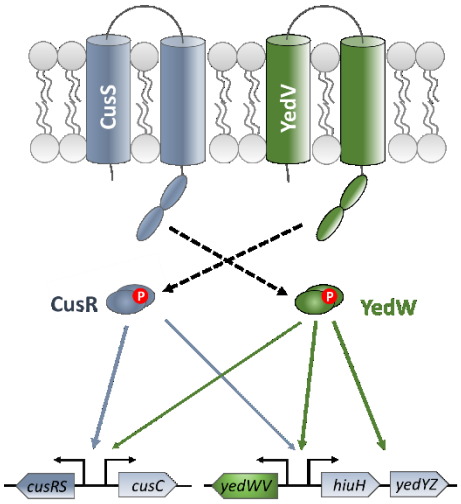

Figure 4.2 Model of CusS/ CusR and YedV/ YedW TCS interconnection based on transcriptomics and promoter activation data. Dashed arrows: assumed cross-phosphorylation between components, solid arrows: regulation of gene expression.

Interestingly the deletion of YedW does not identify any differentially regulated genes whereas the data confirm CusR to be crucial for copper-dependent upregulation of *cusC*, *hiuH* and *yedWV* [53]. The YedV and CusS HKs are involved the signaling of the other TCS by influencing its RR. YedV HK activates the RR CusR though only the transcription of *cusC* is promoted. CusS, if its cognate RR is lacking, mainly influences the transcription of *hiuH*, *yedYZ* and *yedWV*. Dependent on the conditions CusS seems exhibit either an activating or repressing function. In Δ*cusR*$_{MG}$ with native levels of the other TCSs proteins the presence of CusS strongly represses *yedWV* and *hiuH* expression most likely by acting as a phosphatase on the YedW RR. If the YedW RR is present at a higher copy number CusS promotes its effect on gene expression presumably by phosphorylation of the non-cognate RR. Overall our gene expression data supports the assumption of cross-talk of the CusS/ CusR and YedV/ YedW TCSs on the level of HK-RR interaction as well as on promoter level.

5 Conclusion and Outlook

TCSs play an important role in prokaryotic stimulus perception and in the regulation of physiological processes. In this work we studied interactions between cognate and non-cognate components of *E. coli* TCSs and their effect on the overall cell response to a stimulus. Based on stimulus-induced homo-interaction of the HKs CusS, YedV and BaeS we characterized interaction and signaling of the CusS/ CusR, YedV/ YedW and to a lesser extent BaeS/ BaeR TCS in more detail.

The BaeS/ BaeR TCS displayed no involvement in cross-talk reactions with the other two TCSs but we showed interconnection of the CusS/ CusR and the YedV/ YedW TCS on the level of gene gene regulation as well as on the level of non-cognate HK-RR cross-talk.

The overall activation of a known CusR target gene in response to copper was shown to be partially dependent on the YedV/ YedW TCS. We identified cross-talk between YedV-CusR and CusS-YedW based on *in vivo* interactions and gene expression experiments. Both HKs exhibit kinase activity on the non-cognate RRs. For the CusS HK we also observed phosphatase activity on the non-cognate RR. This is possibly dependent on the protein copy number of the YedV/ YedW TCS. A high number of YedV/ YedW proteins causes an amplification of the kinase activity whereas in the absence of its cognate RR and its stimulus copper CusS might act as phosphatase on low abundant YedW. Our data are consistent with findings by Urano *et al.* which have been published recently. This group has found that the CusR and YedW RRs influence the transcription of the same target genes. Among the highly positively influenced genes is the operon of the copper efflux system CusCFBA which shares an intergenic region with the operon of the CusS/ CusR TCS. Also influenced is the transcription of the 5-hydroxyisourate hydrolase gene *hiuH*, the operon of the methionine sulfoxide reductase YedYZ as well as the operon of the YedV/ YedW TCS. The binding of CusR and YedW to the upstream regions of the TCS operons creates an autoregulatory feedback loop of and influenced by the two TCSs. Variations in the transcriptional induction of common target genes and therefore also in protein levels indicate that the signaling by

one of the TCSs does not create the same cell response as signaling of the other TCS. Signaling of the YedV/ YedW TCS causes a more pronounced cell response to oxidative stress by strong induction of *hiuH*, *yedY* and *yedZ* and only a less strong induction of *cusCFBA* transcription. The activation of CusS/ CusR signaling mainly supports the export of copper from the cell by high induction of *cusCFBA* and less pronounced induction of *hiuH* transcription.

We found interconnection and regulatory entanglement of YedV/ YedW with the CusS/ CusR TCS on the level of RR phosphorylation as well as on the level target gene expression. To draw an overall picture of the cooperative signaling of the TCSs the identification of a strong or unique YedV activating stimulus is important. So far our search for a stimulus of the YedV/ YedW TCS was non-conclusive and did not confirm a proposed stimulation by H_2O_2. A protein structure-inferred prediction of YedV binding stimuli was not performed due to insufficient structural information on the periplasmic domain. Therefore an important step would be the determination of its tertiary structure by crystallography. This can provide valuable information on potential stimuli and complete the picture of the induction of the cooperative signaling between the TCSs YedV/ YedW and CusS/ CusR.

6 Literature

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7 Appendix

7.1 Tables

Table 7.1.1 List of chemicals and consumables

	Supplier
Ampicillin sodium salt	ROTH
Bacto tryptone	BD
Bacto yeast extract	BD
Caseinhydrolysate (Casamino acids)	ROTH
Chloramphenicol	ROTH
EDTA (Ethylenediaminetetraacetic acid)	ROTH
Glycerol	ROTH
IPTG (Isopropyl-ß-D-thiogalactopyranoside)	ROTH
Kanamycin sulphate	Sigma-Aldrich
K_2HPO_4	ROTH
KH_2PO_4	ROTH
L-Arabinose	Sigma-Aldrich
$MgSO_4$ x 7 H_2O	Sigma-Aldrich
NaCl	ROTH
$(NH_4)SO_4$	Sigma-Aldrich
Tris base	ROTH
Sodium citrate x 2 H_2O	Sigma-Aldrich

Table 7.1.2 Strains used in this study

Strain	Genotype	Source
E. coli K-12		
MG1655	F^- (λ^-) *rph-1*	[1]
BW25113	*lacI*^{q} *rrnB*$_{T14}$ Δ*lacZ*$_{WJ16}$ *hsdR514* Δ*araBAD*$_{AH33}$ Δ*rhaBAD*$_{LD78}$	[193]
Δ*cusRS*$_{MG}$	*kan*^{r} from JW5082 (Δ*cusS* but a region including the stop codon of upstream *cusR* gene are deleted as well) transduced to MG1655 background and then removed	this work
Δ*cusRS*$_{BW}$	*kan*^{r} removed from JW5082 (Δ*cusS* but a region including the stop codon of upstream *cusR* gene are deleted as well)	[194]
Δ*cusS*$_{251\text{-}1443}$	partial *cusS* knockout in MG 1655 background (upstream *cusR* gene intact)	this work
Δ*cusR*$_{MG}$	*kan*^{r} from JW0560 transduced to MG1655 background and then	this work

Strain *E. coli* K-12	Genotype	Source
	removed	
Δ*cusR*$_{BW}$	*kan*^{r} removed from JW0560	[194]
Δ*yedV*$_{MG}$	*kan*^{r} from JW1951 transduced to MG1655 background and then removed	this work
Δ*yedV*$_{BW}$	*kan*^{r} removed from JW1951	[194]
Δ*yedW*$_{MG}$	*kan*^{r} from JW5322 transduced to MG1655 background and then removed	this work
Δ*yedW*$_{BW}$	*kan*^{r} removed from JW5322	[194]

Table 7.1.3 Plasmids used in this study

Plasmid	Vector	Gene(s)	Cloning sites	Marker	Induction	Source/ reference
pKD13		*rpsB, yfp,kan*^{R}*, tsf*	multiple	amp/ kan		Juliane Winkler (AG Bukau)
pKD46		λ Red		amp	Ara	[193]
pCP20		FLP$^+$		amp/ cam		[196]
pDK112	pTrc99a-RBS	*cheB(1—134)-GTG_YFP*	*Nco*I, *Hind*III	amp	IPTG	D. Kentner
pVS198	pTrc99a		multiple	amp	IPTG	[223], V. Sourjik
pBAD33	-	Expression plasmid	multiple	cam	Ara	[224]
Promoter-GFP fusions						
pAM96	pUA66	*cusC* promoter-GFP fusion	*Xho*I, *BamH*I	kan		[197]
pES174	pUA66	*yedW* promoter-GFP fusion	*Xho*I, *BamH*I	kan		E. Sommer
pMF48	pUA66	*yedX (hiuH)* promoter-GFP fusion	*Xho*I, *BamH*I	kan		[197]
TCSs						
pMF15	pTrc99a-RBS	*cusS*	*Nco*I, *Hind*III	amp	IPTG	this work
pMF16	pTrc99a-RBS	*cusR*	*Nco*I, *Hind*III	amp	IPTG	this work
pMF18	pTrc99a-RBS	*yedV*	*Nco*I, *Hind*III	amp	IPTG	this work
pMF19	pTrc99a-RBS	*yedW*	*Nco*I, *Hind*III	amp	IPTG	this work
pMF23	pTrc99a-RBS	*YedV*$_{H245Q}$ (mutation of conserved His residue)	*Nco*I, *Hind*III	amp	IPTG	this work
pMF29	pTrc99a-	*cpxA*	*Nco*I, *Hind*III	amp	IPTG	this work

Plasmid	Vector	Gene(s)	Cloning sites	Marker	Induction	Source/ reference
pMF30	pTrc99a-RBS	*yedV*$_{N359A,I393A,L399A,F400A}$ (HATPase mutation)	*Nco*I, *Hind*III	amp	IPTG	this work
pMF34	pTrc99a-RBS	*yedWV*	*Nco*I, *Hind*III	amp	IPTG	this work
pMF38	pTrc99a-RBS	*cusS*$_{H176R, H178R}$ (mutation copper binding domain)	*Nco*I, *Hind*III	amp	IPTG	this work
CFP & YFP fusions						
pES75	pBAD33	*baeS-cfp*	*Spe*I, *Hind*III	cam	Ara	E. Sommer
pES81	pBAD33	*cusS-cfp*	*Spe*I, *Hind*III	cam	Ara	E. Sommer
pES149	pBAD33	*yedW-cfp*	*Spe*I, *Hind*III	cam	Ara	E. Sommer
pES87	pBAD33	*yedV-cfp*	*Spe*I, *Hind*III	cam	Ara	E. Sommer
pES101	pBAD33	*arcB-cfp*	*Spe*I, *Hind*III	cam	Ara	E. Sommer
pES97	pBAD33	*barA-cfp*	*Spe*I, *Hind*III	cam	Ara	E. Sommer
pES156	pBAD33	*citA-cfp*	*Spe*I, *Hind*III	cam	Ara	E. Sommer
pES80	pBAD33	*dcuS-cfp*	*Spe*I, *Hind*III	cam	Ara	E. Sommer
pES172	pBAD33	*evgS-cfp*	*Spe*I, *Hind*III	cam	Ara	E. Sommer
pES89	pBAD33	*narX-cfp*	*Spe*I, *Hind*III	cam	Ara	E. Sommer
pES77	pBAD33	*phoR-cfp*	*Spe*I, *Hind*III	cam	Ara	E. Sommer
pES102	pBAD33	*torS-cfp*	*Spe*I, *Hind*III	cam	Ara	E. Sommer
pES76	pBAD33	*uhpB-cfp*	*Spe*I, *Hind*III	cam	Ara	E. Sommer
pES167	pTrc99a	*baeR-yfp* (+100 bp upstream)		amp	IPTG	E. Sommer
pAE1	pTrc99a	*cusS-yfp*	*Nco*I, *BamH*I	amp	IPTG	A. Ernst
pES138	pTrc99a	*cusR-cfp*	*Nco*I, *BamH*I	amp	IPTG	E. Sommer
pES110	pTrc99a	*cusR-yfp*	*Nco*I, *BamH*I	amp	IPTG	E. Sommer
pES135	pTrc99a	*yedW-yfp*	*Nco*I, *BamH*I	amp	IPTG	E. Sommer
pES29	pTrc99a	*yedV-yfp*	*Nco*I, *BamH*I	amp	IPTG	E. Sommer
pES104	pTrc99a	*arcA-yfp*	*Nco*I, *BamH*I	amp	IPTG	E. Sommer
pES122	pTrc99a	*uvry-yfp*	*BspH*I, *Bgl*II	amp	IPTG	E. Sommer
pES112	pTrc99a	*citB-yfp*	*Nco*I, *BamH*I	amp	IPTG	E. Sommer
pES142	pTrc99a	*dcuR-yfp*	*Nco*I, *Bgl*I	amp	IPTG	E. Sommer
pES113	pTrc99a	*evgA-yfp*	*Nco*I, *BamH*I	amp	IPTG	E. Sommer
pES32	pTrc99a	*narQ-yfp*	*Nco*I, *BamH*I	amp	IPTG	E. Sommer
pES166	pTrc99a	*narL-yfp*	*Nco*I, *BamH*I	amp	IPTG	E. Sommer
pES109	pTrc99a	*phoB-yfp*	*Nco*I, *BamH*I	amp	IPTG	E. Sommer
pES107	pTrc99a	*torR-yfp*	*Nco*I, *BamH*I	amp	IPTG	E. Sommer

Table 7.1.4 Primers used in this study

Primer	Nucleotide sequence (5'-3')	Restriction site	Target	Source
ERI22a	ATATACCATGGTGATAGGCAGCTTAACC	*Nco*I	fw primer *cpxA*	[203]
MF57_neu	GCGAAGCTTTTAACTCCGCTTATACAGC	*Hind*III	rev primer *cpxA*	this work
MF96fw2	GCGGAGCTCAACGCAAGCCCGACGCTTAATGACGCTGGAAGATATCGTCAGTGGTTATTGTGTAGGCTGGAGCTGCTTCGAAGTTCCTA	*Sac*I	Fw primer for genomic knockout of Δ*cusS*$_{251-1443}$	this work
MF96rev	GCGAAGCTTGCGGCACGTTATTTTTACACTGGTTATAAAAGTTGCCGTTTGCTGAAGGATGATTCCGGGGATCCGTCGACCTGCAGTTC	*Hind*III	rev primer for genomic knockout of Δ*cusS*$_{251-1443}$	this work
ERI121	CAGTCATAGCCGAATAGCCT	-	verification primer k1 *kan*^{R} cassette knockouts [193]	[203]
ERI122	CGGTGCCCTGAATGAACTGC	-	Verfication primer k2 *kan*^{R} cassette knockouts	[203]
DK101a	ATATACCATGGTCAGTAAGCCATTTC	*Nco*I	fw primer *cusS*	
MF20	GAGAAAGCTTTTAAGCGGGTAATGTGAT	*Hind*III	rev primer *cusS*	this work
ERI67a	TACGACCATGGCAAAACTGTTGATTGTC	*Nco*I	fw primer *cusR*	[203]
MF21	GAGAAAGCTTTTACTGACCATCCGGCAC	*Hind*III	rev primer *cusR*	this work
ERI23a	ATATACCATGGTGAAAAGACTATCTATAACC	*Nco*I	fw primer *yedV*	[203]
MF50a	CGTTCTAAGTTCTTGAGCGAGATCGTC	-	primer YedV$_{H245Q}$ mutation; 1st PCR: ERI23a/ MF50a; 2nd PCR: MF23a/ MF24	this work
MF50b	GACGATCTCGCTCAAGAACTTAGAACG	-	primer YedV$_{H245Q}$ mutation; 1st PCR: MF50b/ MF24; 2nd PCR: ERI23a/ MF24	this work
MF24	GAGAAAGCTTTTAATTTCTTTGCGGTAA	*Hind*III	rev primer *yedV*	this work
ERI69a	TACGACCATGGCAAAGATTCTACTTATTG	*Nco*I	fw primer *yedW*	[203]
MF25	GAGAAAGCTTTTATTTTTTTACCGCTACG	*Hind*III	rev primer *yedW*	this work

Primer	Nucleotide sequence (5'-3')	Restriction site	Target	Source
MF79a	GGAACGAAAGCCAATGAGCCT GAAAAAGCGGCGCGTAGATTTT GGCGGG	-	fw primer *yedV*$_{N359A,I393A,L399A,F400A}$ mutation (HATPase domain)	this work
MF79a rev compl	CCCGCCAAAATCTACGCGCCGC TTTTTCAGGCTCATTGGCTTTCG TTCC	-	rev primer *yedV*$_{N359A,I393A,L399A,F400A}$ mutation (HATPase domain)	this work
MF80a	CTTATTGTTGCCGCCATTAGAT ATTCGC	-	primer *yedV*$_{N359A,I393A,L399A,F400A}$ mutation (HATPase domain)	this work
MF80a rev compl	GCGAATATCTAATGGCGGCAAC AATAAG	-	rev primer *yedV*$_{N359A,I393A,L399A,F400A}$ mutation (HATPase domain)	this work

Table 7.1.5 Linear correlation coefficients of RNA samples of MG1655 RNA sequencing project.

Experiments	R^2
MG1655 pVS198	0.9678
MG1655 YedWV	0.9784
MG1655 YedV	0.9806
MG1655 CusS	0.959
Δ*cusR*$_{MG}$ pVS198	0.9726
Δ*cusR*$_{MG}$ YedWV	0.9484
Δ*cusR*$_{MG}$ YedV	0.9786
Δ*cusR*$_{MG}$ YedW	0.8906
Δ*cusRS*$_{MG}$ pVS198	0.9666
Δ*cusRS*$_{MG}$ YedWV	0.9577
Δ*cusRS*$_{MG}$ YedV	0.983
Δ*cusRS*$_{MG}$ YedW	0.9456
MG1655 pVS198/ Δ*cusRS*$_{MG}$ pVS198	0.9823
MG1655 pVS198/ Δ*cusR*$_{MG}$ pVS198	0.9653
Δ*cusR*$_{MG}$ pVS198/ Δ*cusRS*$_{MG}$ pVS198	0.9826
MG1655 pVS198/ MG1655 YedWV	0.8962
Δ*cusRS*$_{MG}$ pVS198/ Δ*cusRS*$_{MG}$ YedWV	0.9241
Δ*cusR*$_{MG}$ pVS198/ Δ*cusR*$_{MG}$ YedWV	0.9051
MG1655 pVS198/ MG1655 YedV	0.8675
Δ*cusR*$_{MG}$ pVS198/ Δ*cusR*$_{MG}$ YedV	0.914

Δ*cusRS*$_{MG}$ pVS198/ Δ*cusRS*$_{MG}$ YedV	0.8918
Δ*cusR*$_{MG}$ pVS198/ Δ*cusR*$_{MG}$ YedW	0.8116
Δ*cusRS*$_{MG}$ pVS198/ Δ*cusRS*$_{MG}$ YedW	0.854
MG1655 pVS198/ Δ*cusRS*$_{MG}$ YedW	0.8497
MG1655 pVS198/ Δ*cusRS*$_{MG}$ YedV	0.8695
MG1655 pVS198/ Δ*cusR*$_{MG}$ YedW	0.8162
MG1655 pVS198/ Δ*cusR*$_{MG}$ YedV	0.8948
MG1655 pVS198/ MG1766 CusR	0-6234

Table 7.1.6 Linear correlation coefficients of RNA samples of BW25113 RNA sequencing project.

Experiments	R^2
BW25113/ BW25113 $CuSO_4$	0.8419
Δ*cusR*$_{BW}$/ Δ*cusR*$_{BW}$ $CuSO_4$	0.7967
Δ*baeR*$_{BW}$/ Δ*baeR*$_{BW}$ $CuSO_4$	0.8937
Δ*yedW*$_{BW}$/ Δ*yedW*$_{BW}$ $CuSO_4$	0.8146
Δ*cusR*$_{BW}$/ Δ*baeR*$_{BW}$	0.9152
Δ*cusR*$_{BW}$ $CuSO_4$/ Δ*baeR*$_{BW}$ $CuSO_4$	0.7658
BW25113/ Δ*yedW*$_{BW}$	0.8026
BW25113 $CuSO_4$/ Δ*yedW*$_{BW}$ $CuSO_4$	0.9816

Table 7.1.7 4 to 10 fold regulation between MG1655 pVS198 (empty plasmid) and CusR overexpressed in MG1655.

Gene	Annotation	MG1655 CusR
rtcR	sigma 54-dependent transcriptional regulator of rtcBA expression	9.9
yfdV	putative transporter	9.9
puuD	gamma-Glu-GABA hydrolase	9.8
pheP	phenylalanine transporter	9.7
yjcS	conserved protein, metallo-beta-lactamase superfamily	9.7
pgaC	biofilm PGA synthase PgaCD, catalytic subunit; poly-beta-1,6-N-acetyl-D-glucosamine synthase; c-di-GMP-stimulated activity and dimerization	9.6
ygdG	Ssb-binding protein, misidentified as ExoIX	9.6
rrlD	23S ribosomal RNA of rrnD operon	9.6
yrdA		9.5
ybcK	DLP12 prophage, predicted recombinase	9.5
ilvD	dihydroxyacid dehydratase	9.5
metR	DNA-binding transcriptional activator, homocysteine-binding	9.5
citC	[citrate [pro-3S]-lyase] ligase	9.5
yghD	putative secretion pathway M-type protein, membrane anchored	9.4
holD	DNA polymerase III, psi subunit	9.4
purK	N5-carboxyaminoimidazole ribonucleotide synthase	9.2
yagA	CP4-6 prophage, predicted DNA-binding transcriptional regulator	9.1
rrsA	16S ribosomal RNA of rrnA operon	9.1

Gene	Annotation	MG1655 CusR
yagH	CP4-6 prophage, predicted xylosidase/arabinosidase	9.1
torS	hybrid sensory histidine kinase in two-component regulatory system with TorR	8.9
nrfE	heme lyase (NrfEFG) for insertion of heme into c552, subunit NrfE	8.7
rrsC	16S ribosomal RNA of rrnC operon	8.7
xylE	D-xylose transporter	8.7
yehL	putative transporter subunit: ATP-binding component of ABC superfamily	8.6
ybdN		8.6
aslB	putative regulator of arylsulfatase activity	8.5
yncI		8.5
puuB	gamma-Glu-putrescine oxidase, FAD/NAD(P)-binding	8.5
mgtA	magnesium transporter	8.4
nrfF	heme lyase (NrfEFG) for insertion of heme into c552, subunit NrfF	8.4
yfgH	outer membrane integrity lipoprotein	8.3
ybgO	putative fimbrial-like stabilizing protein	8.3
suhB	inositol monophosphatase	8.3
yafW	CP4-6 prophage, antitoxin of the YkfI-YafW toxin-antitoxin system	8.2
rdgC	nucleoid-associated ssDNA and dsDNA binding protein, competitive inhibitor of RecA function	8.1
gfcA		8.1
ybdH	putative oxidoreductase	8.1
hofC	assembly protein in type IV pilin biogenesis, transmembrane protein	8.1
yfcJ	putative arabinose efflux transporter	8
uidC	putative outer membrane porin protein	8
rimI	ribosomal-protein-S18-alanine N-acetyltransferase	8
ilvA	threonine deaminase	8
yehA	putative fimbrial-like adhesin protein	7.9
hyaB	hydrogenase 1, large subunit	7.9
yneK		7.9
yedL	putative acyltransferase	7.9
metI	DL-methionine transporter subunit	7.9
fepE	regulator of length of O-antigen component of lipopolysaccharide chains	7.8
hycC	hydrogenase 3, membrane subunit	7.8
yfbL	putative peptidase	7.7
mutM	formamidopyrimidine/5-formyluracil/ 5-hydroxymethyluracil DNA glycosylase	7.7
ilvE	branched-chain amino-acid aminotransferase	7.7
yifB	putative bifunctional enzyme and transcriptional regulator	7.7
afuB		7.7
ycdT	diguanylate cyclase, membrane-anchored	7.7
rhsE		7.7
ykfG	CP4-6 prophage, predicted DNA repair protein	7.7
yfhR	S9 peptidase family protein, function unknown	7.6
queD	6-pyruvoyl tetrahydrobiopterin synthase (PTPS)	7.5
ybfO		7.5
yhdN	conserved protein, DUF1992 family	7.4

Gene	Annotation	MG1655 CusR
fadB	fused 3-hydroxybutyryl-CoA epimerase/delta(3)-cis-delta(2)-trans-enoyl-CoA isomerase/enoyl-CoA hydratase/3-hydroxyacyl-CoA dehydrogenase	7.4
uhpC	membrane protein regulates uhpT expression	7.4
phnK	carbon-phosphorus lyase complex subunit, putative ATP transporter ATP-binding protein	7.4
ycgX		7.4
rhsD	rhsD element protein	7.3
rrsG	16S ribosomal RNA of rrnG operon	7.3
prfC	peptide chain release factor RF-3	7.3
rrlH	23S ribosomal RNA of rrnH operon	7.3
rrlG	23S ribosomal RNA of rrnG operon	7.2
rrlB	23S ribosomal RNA of rrnB operon	7.2
rrlE	23S ribosomal RNA of rrnE operon	7.2
yehQ		7.1
ygeX	2,3-diaminopropionate ammonia lyase, PLP-dependent	7
yidK	putative transporter	7
ykiA		6.9
ycjX	conserved protein with nucleoside triphosphate hydrolase domain	6.9
yecH	DUF2492 family protein, function unknown	6.9
yibS		6.8
rrlA	23S ribosomal RNA of rrnA operon	6.8
ttdA	L-tartrate dehydratase, alpha subunit	6.7
rhsB	rhsB element core protein RshB	6.7
rlmC	23S rRNA m(5)U747 methyltransferase, SAM-dependent	6.7
proW	glycine betaine transporter subunit	6.7
yjjG	dUMP phosphatase	6.7
glpE	thiosulfate:cyanide sulfurtransferase (rhodanese)	6.7
endA	DNA-specific endonuclease I	6.7
yjaZ	stationary phase growth adaptation protein	6.7
yfcU		6.6
cadA	lysine decarboxylase, acid-inducible	6.6
uhpB	sensory histidine kinase in two-component regulatory system with UhpA	6.6
yrdB		6.6
nrfG	heme lyase (NrfEFG) for insertion of heme into c552, subunit NrfG	6.6
tatD	quality control of Tat-exported FeS proteins, Mg-dependent cytoplasmic DNase	6.6
yhhI	putative transposase	6.6
yjbI		6.6
citF	citrate lyase, citrate-ACP transferase (alpha) subunit	6.6
guaB	IMP dehydrogenase	6.6
ygcN	putative oxidoreductase with FAD/NAD(P)-binding domain	6.6
trmL	tRNA Leu mC34,mU34 2'-O-methyltransferase, SAM-dependent	6.5
folA	dihydrofolate reductase	6.5
recF	gap repair protein	6.5
mhpT	putative 3-hydroxyphenylpropionic transporter	6.5

Gene	Annotation	MG1655 CusR
rlmF	23S rRNA m(6)A1618 methyltransferase, SAM-dependent	6.5
yagB		6.5
yehB	putative outer membrane protein	6.5
mqo	malate dehydrogenase, FAD/NAD(P)-binding domain	6.4
ydeH	diguanylate cyclase, required for pgaD induction	6.4
carA	carbamoyl phosphate synthetase small subunit, glutamine amidotransferase	6.4
metE	5-methyltetrahydropteroyltriglutamate- homocysteine S-methyltransferase	6.4
yjeM	putative transporter	6.4
aaeB	p-hydroxybenzoic acid efflux system component	6.4
yehM		6.4
ttdB	L-tartrate dehydratase, beta subunit	6.4
yfiM	required for high salt suppression of motility, probable lipoprotein	6.4
hsrA	putative multidrug or homocysteine efflux system	6.4
puuP	putrescine importer	6.3
yecF	conserved protein, DUF2594 family	6.3
bglF	fused beta-glucoside-specific PTS enzymes: IIA component/IIB component/IIC component	6.3
ykfH		6.3
puuE	GABA aminotransferase, PLP-dependent	6.3
pgaB	poly-beta-1,6-N-acetyl-D-glucosamine (PGA) N-deacetylase; deacetylase required for biofilm adhesin polysaccharide PGA export; outer membrane lipoprotein	6.3
yjeJ		6.2
insX		6.2
ycgH		6.2
rrrD	DLP12 prophage, predicted lysozyme	6.2
intS	CPS-53 (KpLE1) prophage, predicted prophage CPS-53 integrase	6.2
setC	putative arabinose efflux transporter	6.2
yibJ		6.2
speF	ornithine decarboxylase isozyme, inducible	6.2
pyrE	orotate phosphoribosyltransferase	6.1
yrhC		6.1
arfA	alternate ribosome-rescue factor A	6.1
ydiN	inner membrane protein, predicted MFS superfamily transporter	6.1
ybhI	putative transporter	6.1
glpG	rhomboid intramembrane serine protease	6.1
cusA	copper/silver efflux system, membrane component	6.1
fryA	fused predicted PTS enzymes: Hpr component/enzyme I component/enzyme IIA component	6.1
ykfI	CP4-6 prophage, toxin of the YkfI-YafW toxin-antitoxin system	6.1
zntR	DNA-binding transcriptional activator in response to Zn(II)	6.1
wcaL	putative glycosyl transferase	6
dusC	tRNA-dihydrouridine synthase C	6
yfhL	putative 4Fe-4S cluster-containing protein	6
yfaA	DUF2138 family protein, function unknown	6

Gene	Annotation	MG1655 CusR
phnL	ribophosphonate triphosphate synthase subunit, predicted ATP transporter ATP-binding protein	6
yidD	membrane protein insertion efficiency factor, inner membrane protein, UPF0161 family	5.9
ybbC		5.9
trpE	component I of anthranilate synthase	5.9
rfaH	DNA-binding transcriptional antiterminator	5.9
yliE	putative membrane-anchored cyclic-di-GMP phosphodiesterase	5.8
gltF	periplasmic protein	5.8
yfcC	putative inner membrane protein	5.8
rapA	RNA polymerase-associated helicase protein (ATPase and RNA polymerase recycling factor)	5.8
scpA	methylmalonyl-CoA mutase	5.8
phnE		5.8
proV	glycine betaine transporter subunit	5.8
serB	3-phosphoserine phosphatase	5.8
insK	IS150 transposase B	5.7
lfhA		5.7
ycjW	putative DNA-binding transcriptional regulator	5.7
ssuE	NAD(P)H-dependent FMN reductase	5.7
phnC	phosphonate ABC transporter ATP-binding subunit	5.7
ubiX	3-octaprenyl-4-hydroxybenzoate carboxy-lyase	5.7
dinB	DNA polymerase IV	5.7
yciW	putative oxidoreductase	5.7
htpG	protein refolding molecular co-chaperone Hsp90, Hsp70-dependent; heat-shock protein; ATPase	5.6
argF	ornithine carbamoyltransferase 2, chain F; CP4-6 prophage	5.6
ylbG		5.6
puuR	DNA-binding transcriptional repressor for the puu divergon	5.6
ybbN	DnaK co-chaperone, thioredoxin-like protein	5.6
ybeU	conserved protein, DUF1266 family	5.6
yfdX		5.6
metF	5,10-methylenetetrahydrofolate reductase	5.6
yfiB	putative positive effector of YfiN activity, OM lipoprotein	5.5
glrK	sensor protein kinase regulating glmY sRNA in two-component system with response regulator GlrR	5.5
yqiK	PHB family membrane protein, function unknown	5.5
tsaD	tRNA(ANN) t(6)A37 threonylcarbamoyladenosine modification protein, glycation binding protein	5.5
ibpA	heat shock chaperone	5.5
yieP	putative transcriptional regulator	5.5
kbaY	tagatose 6-phosphate aldolase 1, kbaY subunit	5.5
hslV	peptidase component of the HslUV protease	5.5
yiaD	multicopy suppressor of bamB, outer membrane lipoprotein	5.5
aslA	acrylsulfatase-like enzyme	5.5
potE	putrescine/proton symporter: putrescine/ornithine antiporter	5.5
ygeQ		5.4

Gene	Annotation	MG1655 CusR
crl	sigma factor-binding protein, stimulates RNA polymerase holoenzyme formation	5.4
hyfB	hydrogenase 4, membrane subunit	5.4
yhiN	putative oxidoreductase with FAD/NAD(P)-binding domain	5.4
ydfJ		5.4
yehH		5.4
citE	citrate lyase, citryl-ACP lyase (beta) subunit	5.4
yojI	microcin J25 efflux pump, TolC-dependent; fused ABC transporter permease and ATP-binding components	5.4
yggI		5.4
sgcQ	KpLE2 phage-like element, predicted nucleoside triphosphatase	5.4
yidB	conserved protein, DUF937 family	5.4
recX	regulatory protein for RecA	5.3
alr	alanine racemase 1, PLP-binding, biosynthetic	5.3
ycjF	inner membrane protein, UPF0283 family	5.3
yagG	CP4-6 prophage, predicted sugar transporter	5.3
psiE	phosphate starvation inducible protein	5.2
rrlC	23S ribosomal RNA of rrnC operon	5.2
glpR	DNA-binding transcriptional repressor	5.2
citX	apo-citrate lyase phosphoribosyl-dephospho-CoA transferase	5.2
selU	tRNA 2-selenouridine synthase, selenophosphate-dependent	5.2
sieB	Rac prophage, phage superinfection exclusion protein	5.2
appY	DNA-binding global transcriptional activator, DLP12 prophage	5.2
yphG		5.2
yaeF	putative lipoprotein	5.2
torT	periplasmic sensory protein associated with the TorRS two-component regulatory system	5.2
fimB	tyrosine recombinase/inversion of on/off regulator of fimA	5.1
yegK		5.1
yhcC	putative Fe-S oxidoreductase	5.1
ydjE	putative transporter	5.1
mltF	membrane-bound lytic transglycosylase F, murein hydrolase	5.1
artP	arginine transporter subunit	5.1
yjfK	conserved protein, DUF2491 family	5.1
paaZ	fused oxepin-CoA hydrolase/3-oxo-5,6-dehydrosuberyl-CoA semialdehyde dehydrogenase	5.1
yicI	putative alpha-glucosidase	5.1
corA	magnesium/nickel/cobalt transporter	5.1
ycfZ	inner membrane protein	5
ygjK	alpha-glucosidase	5
trmI	tRNA m(7)G46 methyltransferase, SAM-dependent	5
ydcY		5
sulA	SOS cell division inhibitor	5
codB	cytosine transporter	5
serV	tRNA-Ser	5
yghE		5
rnpA	protein C5 component of RNase P	5

Gene	Annotation	MG1655 CusR
phnM	ribophosphonate triphosphate hydrolase	5
lysC	aspartokinase III	5
frvR	putative frv operon regulator, contains a PTS EIIA domain	4.9
tfaR	Rac prophage, predicted tail fiber assembly protein	4.9
frmB	S-formylglutathione hydrolase	4.9
dnaG	DNA primase	4.9
ybbM	inner membrane protein, UPF0014 family	4.9
entD	phosphopantetheinyltransferase component of enterobactin synthase multienzyme complex	4.9
ynbC	putative hydrolase	4.9
yrbN		4.9
rhlE	ATP-dependent RNA helicase	4.9
borD	DLP12 prophage, predicted lipoprotein	4.9
rpsU	30S ribosomal subunit protein S21	4.9
uidB	glucuronide transporter	4.8
cynR	transcriptional activator of cyn operon, autorepressor	4.8
yfiN	putative membrane-anchored diguanylate cyclase with an N-terminal periplasmic domain	4.8
rsmC	16S rRNA m(2)G1207 methyltransferase, SAM-dependent	4.8
htrE	putative outer membrane usher protein	4.8
dctR	putative DNA-binding transcriptional regulator	4.8
yciV		4.7
hemG	protoporphyrin oxidase, flavoprotein	4.7
yagK	CP4-6 prophage, conserved protein	4.7
ybbO	putative oxidoreductase with NAD(P)-binding Rossmann-fold domain	4.7
tauA	taurine transporter subunit	4.7
stfR	Rac prophage, predicted tail fiber protein	4.7
yfdF		4.7
rzpD	DLP12 prophage, predicted murein endopeptidase	4.7
cusB	copper/silver efflux system, membrane fusion protein	4.7
pgaA	biofilm adhesin polysaccharide PGA secretin; OM porin; poly-beta-1,6-N-acetyl-D-glucosamine export protein	4.7
ebgA	cryptic beta-D-galactosidase, alpha subunit	4.7
yaiP	putative glucosyltransferase	4.6
apt	adenine phosphoribosyltransferase	4.6
yneJ	putative DNA-binding transcriptional regulator	4.6
chiA	periplasmic endochitinase	4.6
pmrR	putative regulator of BasS activity, membrane protein	4.6
gspJ	putative general secretory pathway component, cryptic	4.6
rhaB	rhamnulokinase	4.6
insZ		4.6
ycaI	inner membrane protein, ComEC family of competence proteins	4.6
ybdO	putative DNA-binding transcriptional regulator	4.6
yjiJ	putative inner membrane protein	4.6
emrY	putative multidrug efflux system	4.6
zntA	zinc, cobalt and lead efflux system	4.6
yciQ	putative inner membrane protein	4.5

Gene	Annotation	MG1655 CusR
tyrP	tyrosine transporter	4.5
recQ	ATP-dependent DNA helicase	4.5
uhpA	DNA-binding response regulator in two-component regulatory system with UhpB	4.5
iaaA	Isoaspartyl peptidase	4.5
mhpB	2,3-dihydroxyphenylpropionate 1,2-dioxygenase	4.5
hipB	antitoxin of HipAB toxin-antitoxin system	4.5
ybbA	putative transporter subunit: ATP-binding component of ABC superfamily	4.5
uxuA	mannonate hydrolase	4.5
cysJ	sulfite reductase, alpha subunit, flavoprotein	4.5
mdtA	multidrug efflux system, subunit A	4.5
hyfF	hydrogenase 4, membrane subunit	4.5
miaA	delta(2)-isopentenylpyrophosphate tRNA-adenosine transferase	4.5
yafP	putative acyltransferase with acyl-CoA N-acyltransferase domain	4.4
yedK		4.4
rph	defective ribonuclease PH	4.4
pabC	4-amino-4-deoxychorismate lyase component of para-aminobenzoate synthase multienzyme complex	4.4
pitB	phosphate transporter	4.4
ydgK	inner membrane protein, DUF2569 family	4.4
ycdZ	inner membrane protein, DUF1097 family	4.4
rbfA	30s ribosome binding factor	4.4
prpE	propionate--CoA ligase	4.4
fau	conserved protein, 5-formyltetrahydrofolate cyclo-ligase family	4.4
ybdD	conserved protein, DUF466 family	4.4
ytfA		4.4
yggM		4.4
rluA	dual specificity 23S rRNA pseudouridine(746), tRNA pseudouridine(32) synthase, SAM-dependent	4.3
rsmG	16S rRNA m(7)G527 methyltransferase, SAM-dependent; glucose-inhibited cell-division protein	4.3
yjhR		4.3
radA	DNA repair protein	4.3
prfH		4.3
pyrF	orotidine-5'-phosphate decarboxylase	4.3
fadA	3-ketoacyl-CoA thiolase (thiolase I)	4.3
argA	fused acetylglutamate kinase homolog (inactive)/amino acid N-acetyltransferase	4.3
rtcB		4.3
ygeN		4.3
yhcM	conserved protein with nucleoside triphosphate hydrolase domain	4.3
fimZ	putative DNA-binding transcriptional regulator	4.3
yfjV		4.3
fimD	outer membrane usher protein, type 1 fimbrial synthesis	4.3
csgG	curli production assembly/transport outer membrane lipoprotein	4.2
casA	CRISP RNA (crRNA) containing Cascade antiviral complex protein	4.2

Gene	Annotation	MG1655 CusR
insA	IS1 repressor TnpA	4.2
rpoA	RNA polymerase, alpha subunit	4.2
pagP	phospholipid:lipid A palmitoyltransferase	4.2
yigF	putative inner membrane protein	4.2
hyaA	hydrogenase 1, small subunit	4.2
trkH	potassium transporter	4.2
aegA	fused predicted oxidoreductase: FeS binding subunit/NAD/FAD-binding subunit	4.2
hypB	GTP hydrolase involved in nickel liganding into hydrogenases	4.2
clpB	protein disaggregation chaperone	4.2
mmuM	CP4-6 prophage, S-methylmethionine:homocysteine methyltransferase	4.2
hofB	conserved protein with nucleoside triphosphate hydrolase domain	4.2
tesA	multifunctional acyl-CoA thioesterase I and protease I and lysophospholipase L1	4.2
ydiM	inner membrane protein, predicted transporter	4.2
rpmH	50S ribosomal subunit protein L34	4.1
putA	fused DNA-binding transcriptional regulator/proline dehydrogenase/pyrroline-5-carboxylate dehydrogenase	4.1
uidA	beta-D-glucuronidase	4.1
yhjA	putative cytochrome C peroxidase	4.1
cbl	DNA-binding transcriptional activator for the ssuEADCB and tauABCD operons	4.1
yphH	putative DNA-binding transcriptional regulator	4.1
phnI	ribophosphonate triphosphate synthase complex, probable catalytic subunit	4.1
ybiP	putative hydrolase, inner membrane	4.1
rimP	ribosome maturation factor for 30S subunits	4.1
sdaB	L-serine deaminase II	4.1
bglH	carbohydrate-specific outer membrane porin, cryptic	4.1
ybeT	conserved protein, Sel1 family	4.1
lafU		4
dnaT	DNA biosynthesis protein (primosomal protein I)	4
acrF	multidrug efflux system protein	4
yafU		4
ybjS	putative NAD(P)H-binding oxidoreductase with NAD(P)-binding Rossmann-fold domain	4
gltP	glutamate/aspartate:proton symporter	4
atpI	ATP synthase, membrane-bound accessory factor	4
mnmC	fused 5-methylaminomethyl-2-thiouridine-forming enzyme methyltransferase and FAD-dependent demodification enzyme	4
fimC	periplasmic chaperone	4
gspA	general secretory pathway component, cryptic	4
phoA	bacterial alkaline phosphatase	-4
ppa	inorganic pyrophosphatase	-4
dld	D-lactate dehydrogenase, FAD-binding, NADH independent	-4
ykgF	putative electron transport protein with ferridoxin-like domain	-4
ccmD	cytochrome c biogenesis protein	-4

Gene	Annotation	MG1655 CusR
zapB	FtsZ stabilizer; septal ring assembly factor, stimulates cell division	-4
xylF	D-xylose transporter subunit	-4
dosP	oxygen sensor, c-di-GMP phosphodiesterase, heme-regulated; cold- and stationary phase-induced biofilm regulator	-4.1
prfB	peptide chain release factor RF-2	-4.1
lpp	murein lipoprotein	-4.1
serC	3-phosphoserine/phosphohydroxythreonine aminotransferase	-4.1
sbcD	exonuclease, dsDNA, ATP-dependent	-4.1
csgE	curlin secretion specificity factor	-4.1
hisM	histidine/lysine/arginine/ornithine transporter subunit	-4.1
atoA	acetyl-CoA:acetoacetyl-CoA transferase, beta subunit	-4.1
dmsD	twin-arginine leader-binding protein for DmsA and TorA	-4.1
mlaD	ABC transporter maintaining OM lipid asymmetry, anchored periplasmic binding protein	-4.1
araF	L-arabinose transporter subunit	-4.1
dmlR	DNA-binding transcriptional activator for dmlA	-4.1
alaS	alanyl-tRNA synthetase	-4.2
deoA	thymidine phosphorylase	-4.2
xisD		-4.2
bluF	anti-repressor for YcgE, blue light-responsive; FAD-binding; has c-di-GMP phosphodiesterase-like EAL domain, but does not degrade c-di-GMP	-4.2
aroA	5-enolpyruvylshikimate-3-phosphate synthetase	-4.2
mobB	molybdopterin-guanine dinucleotide biosynthesis protein B	-4.2
xylH	D-xylose ABC transporter permease subunit	-4.2
dgoA	2-oxo-3-deoxygalactonate 6-phosphate aldolase	-4.2
mtlA	fused mannitol-specific PTS enzymes: IIA components/IIB components/IIC components	-4.2
dgoR	putative DNA-binding transcriptional regulator	-4.2
atoD	acetyl-CoA:acetoacetyl-CoA transferase, alpha subunit	-4.2
argT	lysine/arginine/ornithine transporter subunit	-4.2
csrA	pleiotropic regulatory protein for carbon source metabolism	-4.2
hsdR	endonuclease R Type I restriction enzyme	-4.2
katE	catalase HPII, heme d-containing	-4.2
dppD	dipeptide/heme transporter	-4.3
fhuE	ferric-rhodotorulic acid outer membrane transporter	-4.3
yaeP		-4.3
fliG	flagellar motor switching and energizing component	-4.3
acs	acetyl-CoA synthetase	-4.3
lsrB	autoinducer 2-binding protein	-4.3
ydhR	putative monooxygenase	-4.3
ydcA		-4.3
livJ	leucine/isoleucine/valine transporter subunit	-4.3
yniC	2-deoxyglucose-6-P phosphatase	-4.3
gmhA	D-sedoheptulose 7-phosphate isomerase	-4.3
chaA	calcium/sodium:proton antiporter	-4.3
ypjF	CP4-57 prophage, toxin of the YpjF-YfjZ toxin-antitoxin system	-4.3

Gene	Annotation	MG1655 CusR
ftsK	DNA translocase at septal ring sorting daughter chromosomes	-4.3
ydhP	putative transporter	-4.3
yhjX	inner membrane protein, predicted oxalate-formate antiporter	-4.4
gnd	6-phosphogluconate dehydrogenase, decarboxylating	-4.4
yejE	microcin C transporter YejABEF, permease subunit; ABC family	-4.4
ydaU	Rac prophage, conserved protein	-4.4
wbbK	lipopolysaccharide biosynthesis protein	-4.4
tam	trans-aconitate methyltransferase	-4.4
casE	CRISPR RNA precursor cleavage enzyme, CRISP RNA (crRNA) containing Cascade antiviral complex protein	-4.4
dkgA	2,5-diketo-D-gluconate reductase A	-4.4
hisA	N-(5'-phospho-L-ribosyl-formimino)-5-amino-1- (5'-phosphoribosyl)-4-imidazolecarboxamide isomerase	-4.4
ybeL	conserved protein, DUF1451 family	-4.4
galU	glucose-1-phosphate uridylyltransferase	-4.4
csgD	DNA-binding transcriptional activator for csgBA	-4.4
rppH	RNA pyrophosphohydrolase	-4.4
epd	D-erythrose 4-phosphate dehydrogenase	-4.4
ompT	DLP12 prophage, outer membrane protease VII (outer membrane protein 3b)	-4.5
gutM	DNA-binding transcriptional activator of glucitol operon	-4.5
ptsN	sugar-specific enzyme IIA component of PTS	-4.5
hisH	imidazole glycerol phosphate synthase, glutamine amidotransferase subunit with HisF	-4.5
fecI	KpLE2 phage-like element; RNA polymerase, sigma 19 factor	-4.5
ycgR	flagellar velocity braking protein, c-di-GMP-regulated	-4.5
pliG		-4.5
cbpA	curved DNA-binding protein, DnaJ homologue that functions as a co-chaperone of DnaK	-4.5
cohE	e14 prophage, repressor protein phage e14	-4.5
flgE	flagellar hook protein	-4.5
pykF	pyruvate kinase I	-4.6
ptsP	fused PTS enzyme: PEP-protein phosphotransferase (enzyme I)/GAF domain containing protein	-4.6
pal	peptidoglycan-associated outer membrane lipoprotein	-4.6
relB	Qin prophage, bifunctional antitoxin of the RelE-RelB toxin-antitoxin system/ transcriptional repressor	-4.6
pepE	(alpha)-aspartyl dipeptidase	-4.6
sppA	protease IV (signal peptide peptidase)	-4.6
tktB	transketolase 2, thiamin-binding	-4.6
yhfY		-4.6
ribB	3,4-dihydroxy-2-butanone-4-phosphate synthase	-4.6
yiaQ	3-keto-L-gulonate 6-phosphate decarboxylase	-4.6
yobH		-4.6
dcuR	DNA-binding response regulator in two-component regulatory system with DcuS	-4.7
marC	inner membrane protein, UPF0056 family	-4.7

Gene	Annotation	MG1655 CusR
cspB	Qin prophage, cold shock protein	-4.7
ybhP	conserved protein, endo/exonuclease/phosphatase family PFAM PF03372	-4.7
astD	succinylglutamic semialdehyde dehydrogenase	-4.7
sodC	superoxide dismutase, Cu, Zn, periplasmic	-4.7
acuI	putative acryloyl-CoA reductase	-4.7
rutF	flavin:NADH reductase	-4.7
ydjY		-4.7
ybdK	weak gamma-glutamyl:cysteine ligase	-4.7
yaeH	conserved protein, UPF0325 family	-4.8
thiE	thiamin phosphate synthase (thiamin phosphate pyrophosphorylase)	-4.8
psiF	conserved protein, PsiF family, pho regulon	-4.8
agaA		-4.8
bioC	malonyl-CoA methyltransferase, SAM-dependent	-4.8
yoaC	conserved protein, DUF1889 family	-4.8
eutM	putative carboxysome structural protein, ethanolamine utilization protein	-4.8
mdtM	multidrug efflux system protein	-4.8
yheV		-4.8
xylA	D-xylose isomerase	-4.8
yhbJ	glmZ(sRNA)-inactivating NTPase, glucosamine-6-phosphate regulated	-4.9
cyaA	adenylate cyclase	-4.9
paaG	1,2-epoxyphenylacetyl-CoA isomerase, oxepin-CoA-forming	-4.9
tufB	protein chain elongation factor EF-Tu (duplicate of tufA)	-4.9
hisF	imidazole glycerol phosphate synthase, catalytic subunit with HisH	-4.9
yccF	inner membrane protein, DUF307 family	-4.9
tdcA	DNA-binding transcriptional activator	-4.9
gdhA	glutamate dehydrogenase, NADP-specific	-4.9
galE	UDP-galactose-4-epimerase	-4.9
nanA	N-acetylneuraminate lyase	-4.9
yphA	putative inner membrane protein	-5
rnt	ribonuclease T (RNase T)	-5
asr	acid shock-inducible periplasmic protein	-5
yhaH	inner membrane protein, DUF805 family	-5
mqsA	antitoxin for MqsR toxin, transcriptional repressor	-5
ampH	penicillin-binding protein	-5
rhmD	L-rhamnonate dehydratase	-5
paaF	2,3-dehydroadipyl-CoA hydratase	-5
tpx	lipid hydroperoxide peroxidase	-5
manY	mannose-specific enzyme IIC component of PTS	-5
yehY	putative transporter subunit: membrane component of ABC superfamily	-5.1
rcsD	phosphotransfer intermediate protein in two-component regulatory system with RcsBC	-5.1
fucU	L-fucose mutarotase	-5.1
yjiN	zinc-type alcohol dehydrogenase-like protein	-5.1
gatZ	D-tagatose 1,6-bisphosphate aldolase 2, subunit	-5.1
ldrA	toxic polypeptide, small	-5.1

Gene	Annotation	MG1655 CusR
ldrC	toxic polypeptide, small	-5.1
grxA	glutaredoxin 1, redox coenzyme for ribonucleotide reductase (RNR1a)	-5.2
yfaY		-5.2
raiA	cold shock protein associated with 30S ribosomal subunit	-5.2
ymgE	putative inner membrane protein	-5.2
hicB	antitoxin for the HicAB toxin-antitoxin system	-5.2
yneE	putative inner membrane protein, bestrophin family	-5.2
yjiH	putative inner membrane protein	-5.2
pstB	phosphate transporter subunit	-5.2
malF	maltose transporter subunit	-5.2
dppC	dipeptide/heme transporter	-5.3
ushA	bifunctional UDP-sugar hydrolase/5'-nucleotidase	-5.3
yihI	activator of Der GTPase	-5.3
ydcH	DUF465 family protein, function unknown	-5.3
yfcE	phosphodiesterase activity on bis-pNPP	-5.3
patA	putrescine:2-oxoglutaric acid aminotransferase, PLP-dependent	-5.3
dcp	dipeptidyl carboxypeptidase II	-5.3
maeB	fused malic enzyme predicted oxidoreductase/predicted phosphotransacetylase	-5.3
dusB	tRNA-dihydrouridine synthase B	-5.3
potF	putrescine transporter subunit: periplasmic-binding component of ABC superfamily	-5.3
thrA	fused aspartokinase I and homoserine dehydrogenase I	-5.3
livG	leucine/isoleucine/valine transporter subunit	-5.3
ychH	putative inner membrane protein	-5.3
yniA	putative phosphotransferase/kinase	-5.4
yqjE	inner membrane protein, DUF1469 family	-5.4
pepN	aminopeptidase N	-5.4
glpX	fructose 1,6-bisphosphatase II	-5.4
ndk	multifunctional nucleoside diphosphate kinase and apyrimidinic endonuclease and 3'-phosphodiesterase	-5.4
ydaS	Rac prophage, predicted DNA-binding transcriptional regulator	-5.4
ecpA	cryptic Mat fimbrillin gene	-5.4
galT	galactose-1-phosphate uridylyltransferase	-5.4
yncG	glutathione S-transferase homolog	-5.4
rmf	ribosome modulation factor	-5.5
dctA	C4-dicarboxylic acid, orotate and citrate transporter	-5.5
hokD	Qin prophage, small toxic polypeptide	-5.5
torI	response regulator inhibitor for tor operon	-5.5
lamB	maltose outer membrane porin (maltoporin)	-5.5
uspG	universal stress protein UP12	-5.5
yaiY	inner membrane protein, DUF2755 family	-5.5
ygiV	transcriptional repressor for mcbR biofilm gene	-5.5
yjdF	conserved inner membrane protein	-5.6
dppB	dipeptide/heme transporter	-5.6
agaV	N-acetylgalactosamine-specific enzyme IIB component of PTS	-5.6
dsbA	periplasmic protein disulfide isomerase I	-5.6

Gene	Annotation	MG1655 CusR
ftsH	protease, ATP-dependent zinc-metallo	-5.6
ybgS		-5.6
ydfH	putative DNA-binding transcriptional regulator	-5.6
yqiC		-5.6
mobA	molybdopterin-guanine dinucleotide synthase	-5.7
hicA	mRNA interferase toxin of the HicAB toxin-antitoxin system	-5.7
queC	7-cyano-7-deazaguanine (preQ0) synthase, queuosine biosynthesis	-5.7
rpoE	RNA polymerase, sigma 24 (sigma E) factor	-5.7
ydfG	malonic semialdehyde reductase, NADPH-dependent; L-allo-threonine dehydrogenase, NAD(P)-dependent; also oxidizes L-serine, D-serine, D-threonine and 3-hydroxyisobutyrate	-5.8
hemY	putative protoheme IX synthesis protein	-5.8
fbaB	fructose-bisphosphate aldolase class I	-5.8
yjhY		-5.8
yahM		-5.8
yfcG	GSH-dependent disulfide bond oxidoreductase	-5.8
yceH	conserved protein, UPF0502 family	-5.9
bcsF		-5.9
fliJ	flagellar protein	-5.9
ygdD	inner membrane protein, UPF0382 family	-5.9
manX	fused mannose-specific PTS enzymes: IIA component/IIB component	-5.9
thiH	tyrosine lyase, involved in thiamin-thiazole moiety synthesis	-6
tatE	TatABCE protein translocation system subunit	-6
pgk	phosphoglycerate kinase	-6
abgR	putative DNA-binding transcriptional regulator of abgABT operon	-6
nupC	nucleoside (except guanosine) transporter	-6.1
yebK	putative DNA-binding transcriptional regulator	-6.2
yqeF	putative acyltransferase	-6.2
shiA	shikimate transporter	-6.3
talA	transaldolase A	-6.3
potC	polyamine transporter subunit	-6.3
yfeC	putative DNA-binding protein, DUF1323 family	-6.3
rpiR	DNA-binding transcriptional repressor	-6.3
yohO		-6.3
thiF	adenylyltransferase, modifies ThiS C-terminus	-6.3
pgsA	phosphatidylglycerophosphate synthetase	-6.3
dosC	diguanylate cyclase, cold- and stationary phase-induced oxygen-dependent biofilm regulator	-6.4
hemX	putative uroporphyrinogen III methyltransferase	-6.4
rnhA	ribonuclease HI, degrades RNA of DNA-RNA hybrids	-6.4
ytfJ	putative transcriptional regulator	-6.4
lipA	lipoate synthase	-6.5
ydcR	fused predicted DNA-binding transcriptional regulator/predicted amino transferase	-6.5
yhjY		-6.5
mlaB	ABC transporter maintaining OM lipid asymmetry, cytoplasmic STAS component	-6.5

Gene	Annotation	MG1655 CusR
bsmA	biofilm peroxide resistance protein	-6.5
yodD		-6.6
rdoA	Thr/Ser kinase implicated in Cpx stress response	-6.6
trg	methyl-accepting chemotaxis protein III, ribose and galactose sensor receptor	-6.6
lysS	lysine tRNA synthetase, constitutive	-6.6
idnD	L-idonate 5-dehydrogenase, NAD-binding	-6.7
deoD	purine-nucleoside phosphorylase	-6.7
phoU	negative regulator of PhoR/PhoB two-component regulator	-6.7
baeR	DNA-binding response regulator in two-component regulatory system with BaeS	-6.7
manZ	mannose-specific enzyme IID component of PTS	-6.8
yieE	putative phosphopantetheinyl transferase, COG2091 family	-6.8
yedP	putative mannosyl-3-phosphoglycerate phosphatase	-6.8
ddlA	D-alanine-D-alanine ligase A	-6.8
yijD	inner membrane protein, DUF1422 family	-6.9
hisQ	histidine/lysine/arginine/ornithine transporter permease subunit	-6.9
yegP	conserved protein, UPF0339 family	-6.9
aroD	3-dehydroquinate dehydratase	-6.9
rpiB	ribose 5-phosphate isomerase B/allose 6-phosphate isomerase	-6.9
lsrG	autoinducer-2 (AI-2) degrading protein LsrG	-6.9
xthA	exonuclease III	-6.9
rihA	ribonucleoside hydrolase 1	-7
thrB	homoserine kinase	-7
potG	putrescine transporter subunit: ATP-binding component of ABC superfamily	-7
lrp	DNA-binding transcriptional dual regulator, leucine-binding	-7
dnaQ	DNA polymerase III epsilon subunit	-7
livF	leucine/isoleucine/valine transporter subunit	-7
ugpA	glycerol-3-phosphate transporter subunit	-7.1
sdhB	succinate dehydrogenase, FeS subunit	-7.1
focA	formate channel	-7.1
udk	uridine/cytidine kinase	-7.1
bioD	dethiobiotin synthetase	-7.1
aspC	aspartate aminotransferase, PLP-dependent	-7.1
ychN		-7.1
ydbC	putative oxidoreductase, NAD(P)-binding	-7.1
kdgR	DNA-binding transcriptional regulator f kdgK, kdgT, eda	-7.2
patD	gamma-aminobutyraldehyde dehydrogenase	-7.2
chbB	N,N'-diacetylchitobiose-specific enzyme IIB component of PTS	-7.2
osmC	lipoyl-dependent Cys-based peroxidase, hydroperoxide resistance; salt-shock inducible membrane protein; peroxiredoxin	-7.2
yobD	inner membrane protein, UPF0266 family	-7.2
alsE	allulose-6-phosphate 3-epimerase	-7.2
yoaK	expressed protein, membrane-associated	-7.2
sucB	dihydrolipoyltranssuccinase	-7.2
yqjC		-7.2

Gene	Annotation	MG1655 CusR
cbpM	modulator of CbpA co-chaperone	-7.3
sucA	2-oxoglutarate decarboxylase, thiamin-requiring	-7.3
ymfE	e14 prophage, predicted inner membrane protein	-7.3
yfcH	conserved protein with NAD(P)-binding Rossmann-fold domain	-7.3
modF	fused molybdate transporter subunits of ABC superfamily: ATP-binding components	-7.3
amiA	N-acetylmuramoyl-l-alanine amidase I	-7.4
yhjR		-7.4
amn	AMP nucleosidase	-7.5
moaD	molybdopterin synthase, small subunit	-7.5
ynfD		-7.5
yfbU	conserved protein, UPF0304 family	-7.6
yjdJ	putative acyltransferase with acyl-CoA N-acyltransferase domain	-7.6
ybfA		-7.6
mntR	DNA-binding transcriptional regulator of mntH	-7.6
lpd	lipoamide dehydrogenase, E3 component is part of three enzyme complexes	-7.6
ydaV	Rac prophage, predicted DNA replication protein	-7.6
ybhB	kinase inhibitor homolog, UPF0098 family	-7.7
nagB	glucosamine-6-phosphate deaminase	-7.7
thiG	thiamin biosynthesis ThiGH complex subunit	-7.7
bioF	8-amino-7-oxononanoate synthase	-7.8
yqjD	membrane-anchored ribosome-binding protein	-7.8
yrbG	putative calcium/sodium:proton antiporter	-7.8
yciY		-7.8
yghU	putative S-transferase	-7.9
lsrF	putative autoinducer-2 (AI-2) aldolase	-7.9
ydhL		-7.9
nagC	DNA-binding transcriptional dual regulator, repressor of N-acetylglucosamine	-8
yciB	putative inner membrane protein	-8
mipA	scaffolding protein for murein synthesizing machinery	-8
msrC	free methionine-I-sulfoxide reductase	-8
ybgA	conserved protein, DUF1722 family	-8.1
ydgH		-8.1
yfjZ	CP4-57 prophage, antitoxin of the YpjF-YfjZ toxin-antitoxin system	-8.2
pntA	pyridine nucleotide transhydrogenase, alpha subunit	-8.2
thrC	threonine synthase	-8.2
ymdA		-8.3
nagA	N-acetylglucosamine-6-phosphate deacetylase	-8.3
ugpE	glycerol-3-phosphate transporter subunit	-8.3
ydhF	putative oxidoreductase	-8.3
mqsR	GCU-specific mRNA interferase toxin of the MqsR-MqsA toxin-antitoxin system, biofilm/motility regulator, anti-repressor	-8.3
fiu	catecholate siderophore receptor Fiu	-8.4
cspA	RNA chaperone and anti-terminator, cold-inducible	-8.4
sdhC	succinate dehydrogenase, membrane subunit, binds cytochrome b556	-8.4

Gene	Annotation	MG1655 CusR
hdeD	acid-resistance membrane protein	-8.5
serS	seryl-tRNA synthetase, also charges selenocysteinyl-tRNA with serine	-8.5
yeaK		-8.5
yjtD	putative methyltransferase	-8.5
fbaA	fructose-bisphosphate aldolase, class II	-8.5
pdhR	DNA-binding transcriptional dual regulator	-8.5
chrR	chromate reductase, Class I, flavoprotein	-8.6
glnE	fused deadenylyltransferase/adenylyltransferase for glutamine synthetase	-8.6
yciC	inner membrane protein, UPF0259 family	-8.7
aspA	aspartate ammonia-lyase	-8.7
yedW	putative DNA-binding response regulator in two-component system with YedV	-8.7
bioA	7,8-diaminopelargonic acid synthase, PLP-dependent	-8.7
yrdF		-8.7
sucC	succinyl-CoA synthetase, beta subunit	-8.7
hemD	uroporphyrinogen III synthase	-8.7
aroF	3-deoxy-D-arabino-heptulosonate-7-phosphate synthase, tyrosine-repressible	-8.7
yhbY	RNA binding protein associated with pre-50S ribosomal subunits	-8.7
yeiR	Zn-stimulated GTPase involved in zinc homeostasis, mutants are cadmium and EDTA sensitive, Zn(2+) binding protein	-8.8
yjcH	inner membrane protein, DUF485 family	-8.8
ymcE	cold shock gene	-8.8
frdD	fumarate reductase (anaerobic), membrane anchor subunit	-8.9
pgi	glucosephosphate isomerase	-8.9
yfbT	sugar phosphatase	-9
mgsA	methylglyoxal synthase	-9
gstB	glutathione S-transferase	-9
clpX	ATPase and specificity subunit of ClpX-ClpP ATP-dependent serine protease	-9.1
yeaG	protein kinase, function unknown; autokinase	-9.2
ldrD	toxic polypeptide, small	-9.2
ilvH	acetolactate synthase III, stabilizer-dependent, small subunit	-9.2
can	carbonic anhydrase	-9.3
tyrA	fused chorismate mutase T/prephenate dehydrogenase	-9.3
osmF	putative transporter subunit: periplasmic-binding component of ABC superfamily	-9.4
spy	periplasmic ATP-independent protein refolding chaperone, stress-induced	-9.4
proQ	RNA chaperone, probable regulator of ProP translation	-9.6
ydcV	putative spermidine/putrescine transporter subunit	-9.6
fis	global DNA-binding transcriptional dual regulator	-9.6
sucD	succinyl-CoA synthetase, NAD(P)-binding, alpha subunit	-9.8
mglB	methyl-galactoside transporter subunit	-9.9
yeeS	CP4-44 prophage, predicted DNA repair protein	-9.9
agp	glucose-1-phosphatase/inositol phosphatase	-9.9

Gene	Annotation	MG1655 CusR
gabP	gamma-aminobutyrate transporter	-9.9

Table 7.1.8 Genes with fold changes between 4 and 8 and genes of interest between BW25113 and Δ*yedW*$_{BW}$.
Grey: fold change in experiments with $CuSO_4$ and light grey: fold change between experiments without and with $CuSO_4$.

Gene	Annotation	BW25113 → Δ*yedW*$_{BW}$	BW25113 → Δ*yedW*$_{BW}$	BW25113	Δ*yedW*$_{BW}$
purL	phosphoribosylformyl-glycineamide synthetase	-7.9	1.2	-1.1	8.4
purE	N5-carboxyaminoimidazole ribonucleotide mutase	-7.9	1.2	1.2	11.5
ytfT	putative sugar transporter subunit: membrane component of ABC superfamily	-7.5	1.2	-3.2	2.7
nuoF	NADH:ubiquinone oxidoreductase, chain F	-7.4	1.4	-2.1	4.9
glpQ	periplasmic glycerophosphodiester phosphodiesterase	-7.4	2.7	-3.5	5.7
fadH	2,4-dienoyl-CoA reductase, NADH and FMN-linked	-7.3	1.6	-3.9	3.0
purH	fused IMP cyclohydrolase/phosphoribosylaminoimidazole-carboxamide formyltransferase	-7.2	1.2	1.1	9.5
thiP	fused thiamin transporter subunits of ABC superfamily: membrane components	-7.1	1.2	-1.5	5.4
nuoE	NADH:ubiquinone oxidoreductase, chain E	-7.1	1.4	-2.0	5.1
yoeI		-6.9	1.7	1.8	21.3
uraA	uracil permease	-6.8	2.2	-1.5	9.8
nrfD	formate-dependent nitrite reductase, membrane subunit	-6.8	1.1	-4.0	1.9
glpA	sn-glycerol-3-phosphate dehydrogenase (anaerobic), large subunit, FAD/NAD(P)-binding	-6.6	2.4	-2.2	7.2
thiG	thiamin biosynthesis ThiGH complex subunit	-6.3	1.1	1.1	7.2
purM	phosphoribosylaminoimidazole synthetase	-6.2	1.2	-1.1	6.9
rhsB	rhsB element core protein RshB	-6.2	1.1	-4.4	1.5
lyxK	L-xylulose kinase	-6.1	-1.1	-4.5	1.2
gatY	D-tagatose 1,6-bisphosphate aldolase 2, catalytic subunit	-6.0	1.1	-1.4	5.0
sdaC	putative serine transporter	-6.0	1.7	-3.2	3.1
codB	cytosine transporter	-5.9	2.3	-2.1	6.4
fecR	KpLE2 phage-like element, transmembrane signal transducer for ferric citrate transport	-5.8	1.2	-1.3	5.3
fliI	flagellum-specific ATP synthase	-5.8	1.3	-4.7	1.6
aldA	aldehyde dehydrogenase A, NAD-linked	-5.8	1.4	-1.3	6.5
galS	DNA-binding transcriptional repressor	-5.8	2.0	-2.0	6.0
treB	fused trehalose(maltose)-specific PTS enzyme: IIB component/IIC component	-5.7	2.0	-1.7	6.8
fecC	KpLE2 phage-like element, iron-dicitrate	-5.7	1.2	-3.5	1.9

Gene	Annotation	BW25113 → Δ*yedW*$_{BW}$	BW25113 → Δ*yedW*$_{BW}$	BW25113	Δ*yedW*$_{BW}$
	transporter subunit				
ccmF	heme lyase, CcmF subunit	-5.7	1.1	-2.8	2.3
gpsA	glycerol-3-phosphate dehydrogenase (NAD+)	-5.6	1.1	-1.3	5.0
murE	UDP-N-acetylmuramoyl-L-alanyl-D-glutamate:meso- diaminopimelate ligase	-5.6	-1.3	1.5	6.1
mhpB	2,3-dihydroxyphenylpropionate 1,2-dioxygenase	-5.6	1.1	-4.7	1.3
hycD	hydrogenase 3, membrane subunit	-5.6	-1.0	-4.3	1.2
purD	phosphoribosylglycinamide synthetase phosphoribosylamine-glycine ligase	-5.6	1.2	1.1	7.9
tnaA	tryptophanase/L-cysteine desulfhydrase, PLP-dependent	-5.6	2.3	-6.6	1.9
phnI	ribophosphonate triphosphate synthase complex, probable catalytic subunit	-5.5	1.1	-3.6	1.7
xanP	xanthine permease	-5.4	1.1	1.0	6.3
recG	ATP-dependent DNA helicase	-5.4	1.1	-1.5	3.9
oweE		-5.4	-1.2	-1.8	2.5
prpD	2-methylcitrate dehydratase	-5.4	1.3	-5.2	1.3
cusA	nitrate reductase 2 (NRZ), alpha subunit	-5.4	1.1	-3.7	1.5
folK	2-amino-4-hydroxy-6-hydroxymethyldihyropteridine pyrophosphokinase	-5.4	1.1	2.1	12.5
accC	acetyl-CoA carboxylase, biotin carboxylase subunit	-5.4	1.2	-1.1	6.0
prpE	propionate--CoA ligase	-5.4	1.0	-4.0	1.4
bcsG	inner membrane protein, predicted endoglucanase, DUF3260 family	-5.3	-1.2	-1.2	3.7
malF	maltose transporter subunit	-5.3	1.7	-3.6	2.5
rpsA	30S ribosomal subunit protein S1	-5.3	0.0	-1.1	4.9
malE	maltose transporter subunit	-5.3	1.2	-2.8	2.2
hisA	N-(5'-phospho-L-ribosyl-formimino)-5-amino-1-(5'-phosphoribosyl)-4-imidazolecarboxamide isomerase	-5.2	-1.0	1.7	8.8
phnF	putative DNA-binding transcriptional regulator of phosphonate uptake and biodegradation	-5.2	1.0	-2.4	2.2
purF	amidophosphoribosyltransferase	-5.2	1.7	-1.6	5.4
hofP	protein required for the utilization of DNA as a carbon source	-5.2	-1.3	-1.9	2.0
recB	exonuclease V (RecBCD complex), beta subunit	-5.2	1.2	-1.2	5.0
hycC	hydrogenase 3, membrane subunit	-5.2	1.1	-5.3	1.1
mngA	fused 2-O-a-mannosyl-D-glycerate specific PTS enzymes: IIA component/IIB component/IIC component	-5.1	1.2	-4.3	1.4
thiF	adenylyltransferase, modifies ThiS C-terminus	-5.0	-1.1	1.2	5.6
sdhD	succinate dehydrogenase, membrane subunit, binds cytochrome b556	-5.0	1.5	-1.6	4.9

Gene	Annotation	BW25113 → Δ*yedW*$_{BW}$	BW25113 → Δ*yedW*$_{BW}$	BW25113	Δ*yedW*$_{BW}$
yidD	membrane protein insertion efficiency factor, inner membrane protein, UPF0161 family	-5.0	1.7	-1.1	7.4
malT	DNA-binding transcriptional activator for the mal regulon and maltotriose-ATP-binding protein	-5.0	1.5	-1.5	4.8
fecD	KpLE2 phage-like element, iron-dicitrate transporter subunit	-5.0	1.1	-3.7	1.4
purB	adenylosuccinate lyase	-5.0	1.2	-1.0	5.9
fliJ	flagellar protein	-5.0	1.0	-2.6	1.9
pyrD	dihydro-orotate oxidase, FMN-linked	-5.0	1.9	-1.0	9.0
yhfX	putative amino acid racemase	-4.9	-1.0	-3.1	1.6
yqeF	putative acyltransferase	-4.9	1.6	-1.6	4.8
nupC	nucleoside (except guanosine) transporter	-4.9	1.1	-1.4	3.9
rhaD	rhamnulose-1-phosphate aldolase	-4.8	1.2	-3.1	1.9
phnK	carbon-phosphorus lyase complex subunit, putative ATP transporter ATP-binding protein	-4.8	-1.2	-3.0	1.3
yidC	membrane protein insertase	-4.8	1.3	-1.3	4.8
mreC	cell wall structural complex MreBCD transmembrane component MreC	-4.8	1.3	1.3	7.7
yjiS		-4.8	1.2	-2.7	2.1
ygiQ	Radical SAM superfamily protein	-4.7	1.4	-1.4	4.6
yhgF	putative transcriptional accessory protein	-4.7	-1.1	1.1	4.6
narY	nitrate reductase 2 (NRZ), beta subunit	-4.6	-1.0	-2.4	1.8
ytfR	putative sugar transporter subunit: ATP-binding component of ABC superfamily	-4.6	1.2	-2.5	2.2
tauA	taurine transporter subunit	-4.6	-1.0	-3.4	1.3
lhr	putative ATP-dependent helicase	-4.6	-1.0	-2.1	2.1
hisF	imidazole glycerol phosphate synthase, catalytic subunit with HisH	-4.6	1.1	1.7	8.1
nikC	nickel transporter subunit	-4.6	1.1	-2.9	1.7
thiL	thiamin-monophosphate kinase	-4.6	-1.0	-1.0	4.3
pyrF	orotidine-5'-phosphate decarboxylase	-4.5	1.6	-1.1	6.8
glpB	sn-glycerol-3-phosphate dehydrogenase (anaerobic), membrane anchor subunit	-4.5	2.4	-2.2	4.9
phnE		-4.5	-1.0	-3.2	1.4
rbsD	putative cytoplasmic sugar-binding protein	-4.4	1.5	-1.1	5.9
ppx	exopolyphosphatase	-4.4	-1.0	1.0	4.4
tnaB	tryptophan transporter of low affinity	-4.4	1.9	-3.4	2.5
yaaU	putative transporter	-4.4	1.5	-5.0	1.4
hisH	imidazole glycerol phosphate synthase, glutamine amidotransferase subunit with HisF	-4.4	1.0	1.7	7.6
thiQ	thiamin transporter subunit	-4.4	1.1	1.3	6.3
hisD	bifunctional histidinal dehydrogenase/ histidinol dehydrogenase	-4.4	-1.0	1.6	6.8
gudP	putative D-glucarate transporter	-4.4	1.4	-3.9	1.6

Gene	Annotation	BW25113 → Δ*yedW*$_{BW}$	BW25113 → Δ*yedW*$_{BW}$	BW25113	Δ*yedW*$_{BW}$
yddL		-4.4	1.1	-1.6	3.0
yeeN	conserved protein, UPF0082 family	-4.4	-1.2	1.3	5.0
mhpA	3-(3-hydroxyphenyl)propionate hydroxylase	-4.4	1.1	-5.1	-1.1
ykfJ		-4.3	-1.1	-4.9	-1.2
rnb	ribonuclease II	-4.3	1.3	-1.3	4.1
menD	bifunctional 2-oxoglutarate decarboxylase/ SHCHC synthase	-4.3	-1.1	-1.4	2.9
citF	citrate lyase, citrate-ACP transferase (alpha) subunit	-4.3	1.5	-4.5	1.5
paoC	PaoABC aldehyde oxidoreductase, Moco-containing subunit	-4.3	1.0	-3.4	1.3
thiS	immediate sulfur donor in thiazole formation	-4.3	1.0	1.6	6.9
mdtC	multidrug efflux system, subunit C	-4.3	1.1	-1.2	4.0
rhsE		-4.3	1.1	-3.3	1.4
pheA	fused chorismate mutase P/prephenate dehydratase	-4.3	-1.1	1.1	4.0
hybB	putative hydrogenase 2 cytochrome b type component	-4.3	1.2	-3.4	1.5
paaZ	fused oxepin-CoA hydrolase/3-oxo-5,6-dehydrosuberyl-CoA semialdehyde dehydrogenase	-4.2	1.0	-3.2	1.4
rbsA	fused D-ribose transporter subunits of ABC superfamily: ATP-binding components	-4.2	1.9	-1.2	6.4
ttdB	L-tartrate dehydratase, beta subunit	-4.2	1.3	-3.8	1.4
aroA	5-enolpyruvylshikimate-3-phosphate synthetase	-4.2	-1.3	1.7	5.3
tsaB	tRNA(ANN) t(6)A37 threonylcarbamoyladenosine modification protein, binding partner and protease for TsaD	-4.2	1.1	1.1	4.7
yciH	initiation factor function partial mimic, SUI1 family	-4.2	1.3	2.3	12.7
hyfC	hydrogenase 4, membrane subunit	-4.1	1.1	-3.2	1.4
ulaF	L-ribulose 5-phosphate 4-epimerase	-4.1	-1.1	-2.8	1.4
yiaQ	3-keto-L-gulonate 6-phosphate decarboxylase	-4.1	-1.2	-2.0	1.6
hyfD	hydrogenase 4, membrane subunit	-4.1	1.1	-4.1	1.1
oppF	oligopeptide transporter subunit	-4.1	-1.1	-1.4	2.7
cvpA	membrane protein required for colicin V production	-4.1	1.8	-1.4	5.0
fixC	putative oxidoreductase with FAD/NAD(P)-binding domain	-4.1	1.3	-4.0	1.4
yhfZ		-4.1	1.2	1.2	6.0
eutJ	putative chaperonin, ethanolamine utilization protein	-4.1	1.2	-3.3	1.4
ycaO	ribosomal protein S12 methylthiotransferase accessory factor	-4.0	1.2	-1.2	4.2
paaF	2,3-dehydroadipyl-CoA hydratase	-4.0	-1.1	-2.2	1.7

Gene	Annotation	BW25113 → Δ*yedW*$_{BW}$	BW25113 → Δ*yedW*$_{BW}$	BW25113	Δ*yedW*$_{BW}$
ttdA	L-tartrate dehydratase, alpha subunit	-4.0	1.3	-3.5	1.4
yigB	FMN phosphatase	-4.0	-1.0	1.7	6.6
nuoC	NADH:ubiquinone oxidoreductase, fused CD subunit	-4.0	1.4	-2.2	2.7
yffR	CPZ-55 prophage, putative protein	4.0	-1.2	2.3	-2.1
ydhL		4.0	-1.4	1.6	-3.7
flgM	anti-sigma factor for FliA (sigma 28)	4.0	-1.3	1.3	-4.0
hisQ	histidine/lysine/arginine/ornithine transporter permease subunit	4.0	1.3	-1.0	-3.1
yhaL		4.0	-1.1	2.1	-2.2
ybeD	conserved protein, UPF0250 family	4.0	-1.0	2.2	-1.9
yfiL	lipoprotein	4.0	-1.3	1.5	-3.4
nsrR	nitric oxide-sensitive repressor for NO regulon	4.0	-1.2	2.0	-2.4
slyX	protein required for phi X174 lysis	4.1	1.2	2.0	-1.6
yjbJ	stress-induced protein, UPF0337 family	4.1	-1.5	-2.2	-13.8
yifN		4.1	-1.2	2.1	-2.3
blr	beta-lactam resistance membrane protein, divisome-associated protein	4.1	-1.0	2.2	-1.9
ynaE	cold shock protein, function unknown, Rac prophage	4.2	1.0	3.6	-1.1
phoB	DNA-binding response regulator in two-component regulatory system with PhoR (or CreC)	4.2	-1.5	2.7	-2.4
croE	e14 prophage, predicted DNA-binding transcriptional regulator	4.2	-1.1	2.0	-2.3
yiiF		4.2	-1.1	2.0	-2.3
ydhR	putative monooxygenase	4.2	-1.2	1.3	-4.1
ypeC		4.2	-1.1	2.6	-1.9
ychH	putative inner membrane protein	4.2	1.0	-1.6	-6.7
ymgE	putative inner membrane protein	4.3	-1.6	0.0	-6.9
mdtK	multidrug efflux system transporter	4.3	-1.4	-1.0	-6.0
pliG		4.3	-1.4	6.6	1.1
yhcO	putative barnase inhibitor	4.3	-1.6	1.4	-5.0
hokD	Qin prophage, small toxic polypeptide	4.3	-1.0	3.2	-1.4
yfcZ	conserved protein, UPF0381 family	4.3	-1.1	1.2	-4.2
priC	primosomal replication protein N"	4.3	-1.1	1.9	-2.5
sgrT	Inhibitor of glucose uptake	4.4	-1.3	1.9	-2.9
relB	Qin prophage, bifunctional antitoxin of the RelE-RelB toxin-antitoxin system/ transcriptional repressor	4.4	-1.2	2.8	-1.8
uof	ryhB-regulated fur leader peptide	4.4	1.1	2.5	-1.6
glyU	tRNA-Gly	4.4	1.3	2.3	-1.5
yafC	putative DNA-binding transcriptional regulator	4.4	-1.3	2.5	-2.3
ybdF		4.4	-1.2	2.1	-2.6
yjgM	putative acetyltransferase	4.4	-1.1	1.9	-2.7

Gene	Annotation	BW25113 → Δ*yedW*$_{BW}$	BW25113 → Δ*yedW*$_{BW}$	BW25113	Δ*yedW*$_{BW}$
ilvL	ilvG operon leader peptide	4.4	1.1	1.1	-3.6
yhcB		4.4	-1.4	1.4	-4.3
flxA	Qin prophage, putative protein	4.4	-1.1	1.1	-4.6
yhbS	putative acyltransferase with acyl-CoA N-acyltransferase domain	4.4	-1.2	1.1	-4.7
yoaC	conserved protein, DUF1889 family	4.5	-1.7	1.6	-4.7
zapB	FtsZ stabilizer; septal ring assembly factor, stimulates cell division	4.5	-1.3	1.8	-3.2
yoeG		4.5	-1.1	1.7	-3.0
yjbR		4.5	-1.4	1.5	-4.1
ilvN	acetolactate synthase I, small subunit	4.5	1.0	1.2	-3.6
matP	Ter macrodomain organizer matS-binding protein	4.5	-1.0	1.8	-2.6
betI	DNA-binding transcriptional repressor	4.6	-1.3	1.7	-3.3
ydeI		4.6	-1.8	1.1	-7.3
grcA	autonomous glycyl radical cofactor	4.6	-1.7	-1.1	-8.4
pspA	regulatory protein for phage-shock-protein operon	4.6	-1.1	4.0	-1.3
yeaO		4.6	-1.3	2.4	-2.6
ogrK	positive regulator of P2 growth (insertion of P2 ogr gene into the chromosome)	4.6	-1.3	4.2	-1.4
ykgO	RpmJ-like protein	4.7	1.1	20.5	4.7
gadE	DNA-binding transcriptional activator	4.7	-1.5	-2.8	-19.5
ybcW	DLP12 prophage, putative protein	4.7	-1.0	1.5	-3.1
mobA	molybdopterin-guanine dinucleotide synthase	4.7	-1.2	2.3	-2.5
tisB	toxic membrane persister formation peptide, LexA-regulated	4.7	-1.1	2.7	-1.9
ycgZ	RcsB connector protein for regulation of biofilm and acid-resistance	4.7	-1.3	1.8	-3.4
gstA	glutathionine S-transferase	4.8	-1.3	1.2	-5.2
yecT		4.8	-1.1	1.4	-3.7
ybjQ	conserved protein, UPF0145 family	4.8	-1.2	0.0	-5.7
uspD	stress-induced protein	4.8	-1.2	1.1	-5.4
ibsD	toxic membrane protein	4.8	1.3	3.0	-1.2
ibpA	heat shock chaperone	4.8	1.0	5.2	1.1
yagK	CP4-6 prophage, conserved protein	4.8	-1.2	1.7	-3.4
yhjR		4.8	-1.6	2.3	-3.4
ihfB	integration host factor (IHF), DNA-binding protein, beta subunit	4.8	-1.4	1.2	-5.7
uspC	universal stress protein	4.8	-1.2	1.8	-3.3
mntS	Mn(2)-response protein, MntR-repressed	4.9	1.0	1.6	-2.9
yccX	weak acylphosphatase	4.9	-1.3	2.1	-3.1
yafQ	translation inhibitor toxin of toxin-antitoxin pair YafQ/DinJ	4.9	-1.3	3.3	-2.0
raiA	cold shock protein associated with 30S ribosomal	5.0	-1.9	6.7	-1.4

Gene	Annotation	BW25113 → Δ*yedW*$_{BW}$	BW25113 → Δ*yedW*$_{BW}$	BW25113	Δ*yedW*$_{BW}$
	subunit				
fimB	tyrosine recombinase/inversion of on/off regulator of fimA	5.0	-1.6	1.3	-6.2
greB	transcript cleavage factor	5.0	-1.0	1.6	-3.2
nirD	nitrite reductase, NAD(P)H-binding, small subunit	5.0	-1.2	1.5	-3.9
arfA	alternate ribosome-rescue factor A	5.0	-1.3	3.3	-1.9
phoH	conserved protein with nucleoside triphosphate hydrolase domain	5.0	-1.2	1.0	-6.1
ybaM		5.1	-1.3	3.0	-2.1
ygaC		5.2	-1.1	1.3	-4.7
tomB	Hha toxicity attenuator, conjugation-related protein	5.2	-1.4	4.1	-1.7
yiiS	conserved protein, UPF0381 family	5.2	-1.2	1.3	-4.9
ibsC	toxic membrane protein	5.2	2.2	2.2	-1.0
yfcI		5.3	-1.2	1.8	-3.5
yjdJ	putative acyltransferase with acyl-CoA N-acyltransferase domain	5.3	-1.5	1.5	-5.4
mobB	molybdopterin-guanine dinucleotide biosynthesis protein B	5.3	-1.4	3.0	-2.5
ynhF		5.3	1.3	1.9	-2.1
zntR	DNA-binding transcriptional activator in response to Zn(II)	5.3	-1.2	2.3	-2.9
bssR	repressor of biofilm formation by indole transport regulation	5.3	-1.2	2.2	-2.8
yejG		5.3	-1.0	2.1	-2.6
smrA	DNA endonuclease	5.4	1.1	1.3	-3.8
thrW	tRNA-Thr	5.4	1.1	3.0	-1.6
yodD		5.4	-1.5	1.3	-6.4
pspD	peripheral inner membrane phage-shock protein	5.5	-1.1	4.0	-1.5
crl	sigma factor-binding protein, stimulates RNA polymerase holoenzyme formation	5.5	-1.5	1.1	-6.9
ykfN		5.5	-1.3	1.1	-6.8
ybiJ		5.5	-1.6	3.0	-2.8
argD	bifunctional acetylornithine aminotransferase/ succinyldiaminopimelate aminotransferase	5.5	2.1	-2.1	-5.6
yigI	conserved protein, 4HBT family of thioesterases	5.6	-1.1	1.3	-4.7
yhhM	conserved protein, DUF2500 family	5.6	-1.2	2.3	-2.9
yedR	inner membrane protein	5.6	-1.5	1.1	-7.9
rpoH	RNA polymerase, sigma 32 (sigma H) factor	5.7	-1.2	1.6	-4.1
glgS	putative glycogen synthesis protein	5.7	-1.5	-1.0	-8.6
yddM	putative DNA-binding transcriptional regulator	5.7	-1.2	2.7	-2.6
clpS	regulatory protein for ClpA substrate specificity	5.8	-1.2	2.1	-3.3
yhhA	conserved protein, DUF2756 family	5.9	-1.2	1.2	-5.9
yaeR	putative lyase	5.9	-1.2	2.1	-3.4

Gene	Annotation	BW25113 → Δ*yedW*$_{BW}$	BW25113 → Δ*yedW*$_{BW}$	BW25113	Δ*yedW*$_{BW}$
yrbL		5.9	-1.3	-1.6	-12.6
leuL	leu operon leader peptide	6.0	1.1	3.7	-1.5
hisJ	histidine/lysine/arginine/ornithine transporter subunit	6.0	1.2	-1.2	-6.1
pheM	phenylalanyl-tRNA synthetase operon leader peptide	6.0	1.9	2.1	-1.5
yoeH		6.1	-1.5	2.4	-3.8
ybcM	DLP12 prophage, predicted DNA-binding transcriptional regulator	6.1	-1.4	1.9	-4.7
dinJ	antitoxin of YafQ-DinJ toxin-antitoxin system	6.1	-1.2	2.3	-3.2
yaeP		6.2	-1.3	1.2	-6.4
pspC	DNA-binding transcriptional activator	6.4	1.0	3.5	-1.8
ybaQ	putative DNA-binding transcriptional regulator	6.4	-1.2	2.9	-2.6
fucU	L-fucose mutarotase	6.6	-1.3	1.7	-5.1
mgtL	regulatory leader peptide for mgtA	6.6	1.1	4.2	-1.5
rrfE	5S ribosomal RNA of rrnE operon	6.6	1.9	2.6	-1.3
rrfB	5S ribosomal RNA of rrnB operon	6.6	1.9	2.6	-1.3
yrbN		7.0	-1.2	4.3	-2.0
rrfF	5S ribosomal RNA of rrnD operon	7.0	1.6	2.4	-1.9
bolA	stationary-phase morphogene, transcriptional repressor for mreB; also regulator for dacA, dacC, and ampC	7.1	-1.3	-1.3	-11.7
thrL	thr operon leader peptide	7.2	-1.1	5.9	-1.3
soxS	DNA-binding transcriptional dual regulator	7.4	1.1	4.2	-1.6
cnu	oriC-binding complex H-NS/Cnu, binds 26 bp cnb site, also forms a complex with StpA	7.4	-1.4	1.7	-6.4
yoaH		7.4	-1.3	3.1	-3.1
ymcE	cold shock gene	7.8	-1.8	2.9	-4.9
iraP	anti-RssB factor, RpoS stabilizer during Pi starvation; anti-adapter protein	8.0	-1.3	1.2	-9.1

Table 7.1.9 Genes highly affected by YedV expression in MG1655.

Gene	Annotation	MG1655 pVS198 → MG1655 YedV
cusC	putative sensory kinase in two-component regulatory system with YedW	2756.6
cusF	sulfate transporter subunit	1351.9
cusB		915.8
cusA	inner membrane protein, DUF533 family	265.4
yedV	expressed protein, membrane-associated	142.8
metE	inhibitor of the cpx response, periplasmic adaptor protein	41.5
sbp	sulfate adenylyltransferase, subunit 2	40.4
ibpB	pheromone AI-2 transporter	34.0

Gene	Annotation	MG1655 pVS198 → MG1655 YedV
ibpA	periplasmic ATP-independent protein refolding chaperone, stress-induced	24.5
mmuP	5-methyltetrahydropteroyltriglutamate- homocysteine S-methyltransferase	24.2
metL	heat shock chaperone	24.2
yncJ	16S ribosomal RNA of rrnD operon	22.5
metF	sulfate adenylyltransferase, subunit 1	21.3
metB	diguanylate cyclase, required for pgaD induction	20.8
tqsA	heat shock chaperone	16.9
cpxP	thiosulfate-binding protein	16.7
yebE	putative oxidoreductase	15.7
ybdL	L,D-transpeptidase linking Lpp to murein	14.1
yneM	23S ribosomal RNA of rrnH operon	13.5
ycfS	putative endopeptidase	13.1
fxsA	16S ribosomal RNA of rrnH operon	12.1
spy	adenosine 5'-phosphosulfate kinase	11.7
ydeH	putative outer membrane protein	11.4
ycjX	DNA-binding transcriptional activator for the ssuEADCB and tauABCD operons	11.4
acrD	calcium/sodium:proton antiporter	11.1
htpX	16S ribosomal RNA of rrnC operon	10.7
pheP	23S ribosomal RNA of rrnA operon	10.6
chaA	16S ribosomal RNA of rrnA operon	10.6
iraM	putative fimbrial-like adhesin protein	9.7
ydeS	sulfate/thiosulfate ABC transporter permease	9.3
clpB	23S ribosomal RNA of rrnC operon	8.9
ydeR	protein disaggregation chaperone	8.9
cysD	sulfite reductase, alpha subunit, flavoprotein	8.9
rrsD	Hha toxicity attenuator, conjugation-related protein	8.8
ybdM		8.7
bhsA	16S ribosomal RNA of rrnG operon	8.6
cysN	peripheral inner membrane phage-shock protein	8.6
metA	aminoglycoside/multidrug efflux system	8.5
ycjF	conserved protein with nucleoside triphosphate hydrolase domain	8.5
cysP	23S ribosomal RNA of rrnD operon	8.5
pspD	cold shock protein associated with 30S ribosomal subunit	8.2
cysU	RpoS stabilizer during Mg starvation, anti-RssB factor	8.1
yciW	sulfite reductase, beta subunit, NAD(P)-binding, heme-binding	8.1
flgE	galactitol-specific enzyme IIB component of PTS	-8.0
katE	glucitol/sorbitol-specific enzyme IIA component of PTS	-8.1
intG	deferrochelatase, periplasmic	-8.3
glpQ		-8.4
ycaC		-8.5
yciF	putative DNA-binding response regulator in two-component system with YedV	-8.6
glpT	inactive ferrous ion transporter EfeUOB	-8.6
ydcT	fused maltose transport subunit, ATP-binding component of ABC	-8.8

Gene	Annotation	MG1655 pVS198 → MG1655 YedV
	superfamily/regulatory protein	
lamB	periplasmic glycerophosphodiester phosphodiesterase	-9.0
poxB	maltose transporter subunit	-9.1
ydcS	galactitol-specific enzyme IIC component of PTS	-9.2
malK	outer membrane porin 1a (Ia, b, F)	-10.0
yahO	carbamoyl phosphate synthetase small subunit, glutamine amidotransferase	-10.5
efeO	CP4-44 prophage, predicted membrane protein	-10.7
gatD	sn-glycerol-3-phosphate transporter	-10.8
aldB	carbamoyl-phosphate synthase large subunit	-11.1
yagU	galactitol-1-phosphate dehydrogenase, Zn-dependent and NAD(P)-binding	-12.0
malE	aspartate carbamoyltransferase, regulatory subunit	-12.5
	CP4-44 prophage, antigen 43 (Ag43) phase-variable biofilm formation	
ompF	autotransporter	-15.1
fliC	aspartate carbamoyltransferase, catalytic subunit	-17.8

Table 7.1.10 Fold changes more than 4- fold between MG1655 pVS198 and Δ*cusR*$_{MG}$ pVS198.

Gene	Annotation	Δ*cusR*$_{MG}$	Δ*cusRS*$_{MG}$
	putative sensory kinase in two-component regulatory system with		
yedV	YedW	-53.9	3.8
	CP4-44 prophage, antigen 43 (Ag43) phase-variable biofilm formation		
flu	autotransporter	-33.7	-39.8
	putative DNA-binding response regulator in two-component system		
yedW	with YedV	-23.5	7.4
yeeR	CP4-44 prophage, predicted membrane protein	-15.9	-15.2
hiuH	hydroxyisourate hydrolase	-7.4	7.0
	carbamoyl phosphate synthetase small subunit, glutamine		
carA	amidotransferase	-5.1	-1.5
xanP	xanthine permease	-5.0	-1.7
yeeS	CP4-44 prophage, predicted DNA repair protein	-4.6	-6.9
codB	cytosine transporter	-4.4	-1.6
iaaA	Isoaspartyl peptidase	4.1	2.2
iraD	RpoS stabilizer after DNA damage, anti-RssB factor	4.1	2.3
csgB	curlin nucleator protein, minor subunit in curli complex	4.1	-1.2
yiiX	putative lipid binding hydrolase, DUF830 family, function unknown	4.2	2.4
ysgA	putative hydrolase	4.4	2.3
	DNA-binding transcriptional activator for the ssuEADCB and		
cbl	tauABCD operons	4.4	2.3
metJ	DNA-binding transcriptional repressor, S-adenosylmethionine-binding	4.4	2.6
yeeE	inner membrane protein, UPF0394 family	4.4	2.2
cysA	sulfate/thiosulfate transporter subunit	4.6	2.0
metC	cystathionine beta-lyase, PLP-dependent	4.7	3.0
cysH	3'-phosphoadenosine 5'-phosphosulfate reductase	4.8	2.4
metR	DNA-binding transcriptional activator, homocysteine-binding	5.0	4.8

Gene	Annotation	Δ*cusR*$_{MG}$	Δ*cusRS*$_{MG}$
cysW	sulfate/thiosulfate ABC transporter subunit	5.1	2.2
cysI	sulfite reductase, beta subunit, NAD(P)-binding, heme-binding	5.4	2.3
ygbE	DUF3561 family inner membrane protein	5.5	2.4
metI	DL-methionine transporter subunit	6.2	3.9
metN	DL-methionine transporter subunit	6.4	3.8
cysU	sulfate/thiosulfate ABC transporter permease	6.5	2.8
ybdH	putative oxidoreductase	7.0	4.4
cysJ	sulfite reductase, alpha subunit, flavoprotein	7.0	2.9
mmuM	CP4-6 prophage, S-methylmethionine:homocysteine methyltransferase	7.0	3.9
ybdM		7.4	5.3
cysC	adenosine 5'-phosphosulfate kinase	7.4	3.0
metQ	DL-methionine transporter subunit	7.6	4.2
yciW	putative oxidoreductase	8.0	3.4
sbp	sulfate transporter subunit	8.7	3.7
cysP	thiosulfate-binding protein	9.1	3.5
cysN	sulfate adenylyltransferase, subunit 1	9.5	3.5
metA	homoserine O-transsuccinylase	10.1	5.6
cysD	sulfate adenylyltransferase, subunit 2	11.6	4.2
ybdL	methionine aminotransferase, PLP-dependent	15.8	9.1
metF	5,10-methylenetetrahydrofolate reductase	21.0	10.7
mmuP	CP4-6 prophage, predicted S-methylmethionine transporter	21.6	11.0
metB	cystathionine gamma-synthase, PLP-dependent	22.0	12.1
metL	fused aspartokinase II/homoserine dehydrogenase II	23.1	10.1
metE	5-methyltetrahydropteroyltriglutamate- homocysteine S-methyltransferase	41.5	41.5

Table 7.1.11 Fold changes between 4 and 8 fold in BW25113 and Δ*cusR*$_{BW}$ without and with $CuSO_4$.
Table sorted according to the fold changes in BW25113.

Gene	Annotation	BW25113 → BW25113 $CuSO_4$	Δ*cusR*$_{BW}$ → Δ*cusR*$_{BW}$ $CuSO_4$
cueO	multicopper oxidase (laccase)	7.4	12.8
chaA	calcium/sodium:proton antiporter	7.4	3.4
raiA	cold shock protein associated with 30S ribosomal subunit	6.7	4.5
ycfS	L,D-transpeptidase linking Lpp to murein	6.7	1.0
degP	serine endoprotease (protease Do), membrane-associated	6.6	1.8
yhdV	putative outer membrane protein	6.6	-1.6
pliG		6.6	6.0
serT	tRNA-Ser	6.3	1.8
cusS	sensory histidine kinase in two-component regulatory system with CusR, senses copper ions	6.1	1.9
thrL	thr operon leader peptide	5.9	1.1
rrsA	16S ribosomal RNA of rrnA operon	5.7	-1.6
pspG	phage shock protein G	5.6	-1.4

Gene	Annotation	BW25113 → BW25113 $CuSO_4$	Δ*cusR*$_{BW}$ → Δ*cusR*$_{BW}$ $CuSO_4$
mgrB	regulatory peptide for PhoPQ, feedback inhibition	5.6	1.1
yfeK		5.3	-1.9
ibpA	heat shock chaperone	5.2	2.6
ygiM	SH3 domain protein	5.0	-2.2
pspB	DNA-binding transcriptional regulator of psp operon	5.0	-1.1
yhcN		5.0	2.2
ftnB	ferritin B, probable ferrous iron reservoir	5.0	3.8
trpA	tryptophan synthase, alpha subunit	4.9	4.3
nrdI	flavodoxin required for NrdEF cluster assembly	4.7	8.9
trpC	fused indole-3-glycerolphosphate synthetase/N-(5-phosphoribosyl)anthranilate isomerase	4.7	3.1
valW	tRNA-Val	4.6	-4.5
trpB	tryptophan synthase, beta subunit	4.6	3.5
ilvX		4.5	2.9
hisL	his operon leader peptide	4.5	1.3
yneM	expressed protein, membrane-associated	4.5	4.7
nrdH	hydrogen donor for NrdEF electron transport system, glutaredoxin-like protein	4.3	10.3
ibpB	heat shock chaperone	4.3	1.2
yrbN		4.3	-2.5
ogrK	positive regulator of P2 growth (insertion of P2 ogr gene into the chromosome)	4.2	-1.5
soxS	DNA-binding transcriptional dual regulator	4.2	8.7
ydiE	hemin uptake protein HemP homolog	4.2	-2.0
pmrR	putative regulator of BasS activity, membrane protein	4.2	-2.1
mgtL	regulatory leader peptide for mgtA	4.2	-1.2
ydeH	diguanylate cyclase, required for pgaD induction	4.1	2.9
tomB	Hha toxicity attenuator, conjugation-related protein	4.1	2.6
rrsC	16S ribosomal RNA of rrnC operon	4.0	-1.5
znuC	zinc transporter subunit: ATP-binding component of ABC superfamily	4.0	1.5
pspA	regulatory protein for phage-shock-protein operon	4.0	1.0
pspD	peripheral inner membrane phage-shock protein	4.0	-1.4
trpD	fused glutamine amidotransferase (component II) of anthranilate synthase/anthranilate phosphoribosyl transferase	4.0	3.5
trpE	component I of anthranilate synthase	4.0	2.8
ebgA	cryptic beta-D-galactosidase, alpha subunit	-4.0	4.6
araD	L-ribulose-5-phosphate 4-epimerase	-4.0	4.6
prpE	propionate--CoA ligase	-4.0	6.6
fixC	putative oxidoreductase with FAD/NAD(P)-binding domain	-4.0	4.5
sdaB	L-serine deaminase II	-4.0	-3.8
ygjK	alpha-glucosidase	-4.0	5.0
osmY	periplasmic protein	-4.0	-3.0
nrfD	formate-dependent nitrite reductase, membrane subunit	-4.0	9.9
yjiC		-4.0	2.8

Gene	Annotation	BW25113 → BW25113 $CuSO_4$	Δ*cusR*$_{BW}$ → Δ*cusR*$_{BW}$ $CuSO_4$
kdpA	potassium translocating ATPase, subunit A	-4.0	6.0
rhsA	rhsA element core protein RshA	-4.0	8.4
lamB	maltose outer membrane porin (maltoporin)	-4.0	1.3
yhhH		-4.1	3.6
hyfD	hydrogenase 4, membrane subunit	-4.1	6.5
yehM		-4.1	5.4
ytjA		-4.1	-3.9
slp	outer membrane lipoprotein	-4.1	-1.7
fecA	KpLE2 phage-like element, ferric citrate outer membrane transporter	-4.2	1.7
flhB	flagellin export apparatus, substrate specificity protein	-4.2	1.6
mngA	fused 2-O-a-mannosyl-D-glycerate specific PTS enzymes: IIA component/IIB component/IIC component	-4.3	7.3
hycD	hydrogenase 3, membrane subunit	-4.3	8.4
ybfQ		-4.3	1.3
yiaM	L-dehydroascorbate transporter, TRAP permease small subunit for TRAP (TRipartite ATP-independent Periplasmic) family transporter YiaMNO	-4.4	2.9
yphG		-4.4	8.5
ytcA		-4.4	2.3
rhsB	rhsB element core protein RshB	-4.4	10.6
citF	citrate lyase, citrate-ACP transferase (alpha) subunit	-4.5	4.2
lyxK	L-xylulose kinase	-4.5	10.0
tap	methyl-accepting protein IV	-4.6	-3.7
mhpB	2,3-dihydroxyphenylpropionate 1,2-dioxygenase	-4.7	4.4
fliI	flagellum-specific ATP synthase	-4.7	-2.3
ykfJ		-4.9	1.6
fliF	flagellar basal-body MS-ring and collar protein	-4.9	-1.6
yaaU	putative transporter	-5.0	6.7
yagU	inner membrane protein, DUF1440 family	-5.0	-1.4
fdrA	multicopy suppressor of dominant negative ftsH mutations, predicted acyl-CoA synthetase with NAD(P)-binding Rossmann-fold domain	-5.0	5.6
mhpA	3-(3-hydroxyphenyl)propionate hydroxylase	-5.1	8.3
prpD	2-methylcitrate dehydratase	-5.2	6.0
zntA	zinc, cobalt and lead efflux system	-5.2	1.5
fimA	major type 1 subunit fimbrin (pilin)	-5.3	-6.4
hycC	hydrogenase 3, membrane subunit	-5.3	9.2
fliC	flagellar filament structural protein (flagellin)	-5.9	-3.5
hdeD	acid-resistance membrane protein	-5.9	1.2
tar	methyl-accepting chemotaxis protein II	-6.5	-2.1
tnaA	tryptophanase/L-cysteine desulfhydrase, PLP-dependent	-6.6	-2.1
gadC	glutamate:gamma-aminobutyric acid antiporter	-7.5	1.0

Table 7.1.12 Fold change more than 10 between MG1655 pVS198 and MG1655 CusR. The highest 15 regulated genes are not depicted in this table but in Table 3.3.3.

Gene	Annotation	MG1655 CusR
yhiL		16.6
yebB	conserved protein, DUF830 family	16.6
yagI	CP4-6 prophage, predicted DNA-binding transcriptional regulator	16.3
yqiH	putative periplasmic pilin chaperone	16.1
yjjQ	DNA-binding transcriptional regulator	16.0
ykgH	putative inner membrane protein	15.7
ybdL	methionine aminotransferase, PLP-dependent	14.8
ycdU	putative inner membrane protein	14.8
ilvG		14.6
mmuP	CP4-6 prophage, predicted S-methylmethionine transporter	14.5
ydeT		13.7
yfgI		13.6
metN	DL-methionine transporter subunit	13.4
ybdM		13.3
yjgN	inner membrane protein, DUF898 family	13.3
ttdT	L-tartrate/succinate antiporter	13.0
ylbH		12.9
ygaY		12.9
ybcF	putative carbamate kinase	12.6
yhbX	putative hydrolase, inner membrane	12.6
yjbM		12.6
ygeH	predicted transcriptional regulator	12.2
xanP	xanthine permease	11.8
afuC	CP4-6 prophage, predicted ferric transporter subunit	11.8
puuA	gamma-Glu-putrescine synthase	11.8
yigE	putative protein, DUF2233 family	11.7
ygaQ		11.5
yqiI		11.3
rrsH	16S ribosomal RNA of rrnH operon	11.3
yqiG		11.2
ompN	outer membrane pore protein N, non-specific	11.1
yraI	putative periplasmic pilin chaperone	11.1
yfdE	putative CoA-transferase, NAD(P)-binding	11.0
iraD	RpoS stabilizer after DNA damage, anti-RssB factor	11.0
yhhZ		10.9
yaiX		10.8
ibpB	heat shock chaperone	10.7
puuC	gamma-Glu-gamma-aminobutyraldehyde dehydrogenase, NAD(P)H-dependent	10.7
cadB	putative lysine/cadaverine transporter	10.6
yhiM	acid resistance protein, inner membrane	10.5
gltS	glutamate transporter	10.5
yjfJ	conserved protein, PspA/IM30 family	10.5
sfmD	putative outer membrane export usher protein	10.4
yiaN	L-dehydroascorbate transporter, TRAP permease large subunit for TRAP (Tripartite	10.4

Gene	Annotation	MG1655 CusR
	ATP-independent Periplasmic) family transporter YiaMNO	
yibD	putative glycosyl transferase	10.3
purE	N5-carboxyaminoimidazole ribonucleotide mutase	10.2
ygjV	inner membrane protein, Imp-YgjV family	-10.0
pntB	pyridine nucleotide transhydrogenase, beta subunit	-10.0
yibL	conserved protein, ribosome-associated	-10.1
agaW		-10.2
ugpB	glycerol-3-phosphate transporter subunit	-10.2
uof	ryhB-regulated fur leader peptide	-10.2
dppA	dipeptide transporter	-10.2
ydbL		-10.3
ycaP	putative inner membrane protein, UPF0702 family	-10.3
rraB	protein inhibitor of Rnase E	-10.3
uxaA	altronate hydrolase	-10.4
modE	DNA-binding transcriptional repressor for the molybdenum transport operon modABC	-10.5
osmY	periplasmic protein	-10.5
cspD	inhibitor of DNA replication, cold shock protein homolog	-10.6
yahK	putative oxidoreductase, Zn-dependent and NAD(P)-binding	-10.6
ynbE	Lipoprotein	-10.6
ynfN	Qin prophage, cold shock-induced protein	-10.7
ymdF		-10.8
yfcF	glutathione S-transferase	-10.9
frdA	fumarate reductase (anaerobic) catalytic and NAD/flavoprotein subunit	-11.0
npr	phosphohistidinoprotein-hexose phosphotransferase component of N-regulated PTS system (Npr)	-11.0
prc	carboxy-terminal protease for penicillin-binding protein 3	-11.0
panB	3-methyl-2-oxobutanoate hydroxymethyltransferase	-11.1
poxB	pyruvate dehydrogenase (pyruvate oxidase), thiamin-dependent, FAD-binding	-11.1
elbB	isoprenoid biosynthesis protein with amidotransferase-like domain	-11.3
nudK	GDP-mannose pyrophosphatase	-11.3
yebV		-11.4
bioB	biotin synthase	-11.4
glgS	putative glycogen synthesis protein	-11.4
chaB	cation transport regulator	-11.4
znuB	zinc transporter subunit: membrane component of ABC superfamily	-11.5
hiuH	hydroxyisourate hydrolase	-11.5
ymdB	O-acetyl-ADP-ribose deacetylase, Rnase III inhibitor during cold shock, putative cardiolipin synthase C regulatory subunit	-11.5
gabT	4-aminobutyrate aminotransferase, PLP-dependent	-11.7
malK	fused maltose transport subunit, ATP-binding component of ABC superfamily/regulatory protein	-11.8
yidQ	conserved outer membrane protein	-11.9
mdh	malate dehydrogenase, NAD(P)-binding	-11.9
sodA	superoxide dismutase, Mn	-11.9
aldA	aldehyde dehydrogenase A, NAD-linked	-11.9

Gene	Annotation	MG1655 CusR
yegH	inner membrane protein	-12.1
dhaL	dihydroxyacetone kinase, C-terminal domain	-12.1
hpt	hypoxanthine phosphoribosyltransferase	-12.2
ompW	outer membrane protein W	-12.2
hisJ	histidine/lysine/arginine/ornithine transporter subunit	-12.3
idi	isopentenyl diphosphate isomerase	-12.3
potD	polyamine transporter subunit	-12.3
greA	transcript cleavage factor	-12.4
fruB	fused fructose-specific PTS enzymes: IIA component/HPr component	-12.4
ydcS	polyhydroxybutyrate (PHB) synthase, ABC transporter periplasmic binding protein homolog	-12.5
mglC	methyl-galactoside transporter subunit	-12.6
trxB	thioredoxin reductase, FAD/NAD(P)-binding	-12.6
thiS	immediate sulfur donor in thiazole formation	-12.7
dcuA	C4-dicarboxylate antiporter	-12.8
ndh	respiratory NADH dehydrogenase 2/cupric reductase	-12.8
yfbR	5‘-nucleotidase	-12.8
folX	D-erythro-7,8-dihydroneopterin triphosphate 2’-epimerase and dihydroneopterin aldolase	-12.9
djlA	DnaJ-like protein, membrane anchored	-13.0
pdxK	pyridoxal-pyridoxamine kinase/hydroxymethylpyrimidine kinase	-13.1
fliC	flagellar filament structural protein (flagellin)	-13.1
cspE	DNA-binding transcriptional repressor	-13.1
ydcT	putative spermidine/putrescine transporter subunit	-13.2
uxaC	uronate isomerase	-13.2
nagE	fused N-acetyl glucosamine specific PTS enzyme: IIC, IIB, and IIA components	-13.3
ygdR	putative lipoprotein	-13.6
pykA	pyruvate kinase II	-13.7
aceF	pyruvate dehydrogenase, dihydrolipoyltransacetylase component E2	-13.8
lptD	LPS assembly OM complex LptDE, beta-barrel component	-13.8
iraP	anti-RssB factor, RpoS stabilizer during Pi starvation; anti-adapter protein	-14.2
surA	peptidyl-prolyl cis-trans isomerase (PPIase)	-14.5
yeeR	CP4-44 prophage, predicted membrane protein	-14.7
sdhA	succinate dehydrogenase, flavoprotein subunit	-14.8
leuL	leu operon leader peptide	-14.8
yeaD		-14.9
frdB	fumarate reductase (anaerobic), Fe-S subunit	-15.0
clpP	proteolytic subunit of ClpA-ClpP and ClpX-ClpP ATP-dependent serine proteases	-15.6
dps	Fe-binding and storage protein, stress-inducible DNA-binding protein	-15.7
cspI	Qin prophage, cold shock protein	-15.7
hemC	hydroxymethylbilane synthase	-15.7
yfeZ	inner membrane protein	-15.7
arcA	DNA-binding response regulator in two-component regulatory system with ArcB or CpxA	-15.8
frdC	fumarate reductase (anaerobic), membrane anchor subunit	-15.9
ytfK	conserved protein, DUF1107 family	-16.2

Gene	Annotation	MG1655 CusR
icd	e14 prophage; isocitrate dehydrogenase, specific for NADP+	-16.4
pck	phosphoenolpyruvate carboxykinase	-16.4
nmpC		-16.5
yaiZ	putative inner membrane protein, DUF2754 family	-17.0
aceE	pyruvate dehydrogenase, decarboxylase component E1, thiamin-binding	-17.7
acrZ	AcrAB-TolC efflux pump accessory protein, membrane-associated	-17.8
yceB	lipoprotein, DUF1439 family	-18.2
sdhD	succinate dehydrogenase, membrane subunit, binds cytochrome b556	-18.3
aldB	aldehyde dehydrogenase B	-18.4
eno	Enolase	-18.4
csgA	curlin subunit, amyloid curli fibers, cryptic	-18.4
yeaQ	conserved protein, UPF0410 family	-18.6
ygdI	putative lipoprotein	-18.8
exbB	membrane spanning protein in TonB-ExbB-ExbD complex	-18.9
pflB	pyruvate formate lyase I	-18.9
ygiF	putative adenylate cyclase	-19.0
sra	stationary-phase-induced ribosome-associated protein	-19.1
yciI	putative enzyme	-19.5
lhgO	L-2-hydroxyglutarate oxidase	-19.5
yihD	DUF1040 protein YihD	-20.1
yjjY		-20.4
fruK	fructose-1-phosphate kinase	-20.8
hdeA	stress response protein acid-resistance protein	-21.5
ydiZ		-21.6
ygiW		-21.7
gpmA	phosphoglyceromutase 1	-22.1
exbD	membrane spanning protein in TonB-ExbB-ExbD complex	-22.3
zwf	glucose-6-phosphate 1-dehydrogenase	-23.2
topB	DNA topoisomerase III	-23.2
ykgL		-24.0
mtfA	anti-repressor for DgsA(Mlc)	-25.2
tonB	membrane spanning protein in TonB-ExbB-ExbD transport complex	-25.3
yahO	periplasmic protein, function unknown, YhcN family	-26.0
yoaB	putative reactive intermediate deaminase	-26.1
hdeB	acid-resistance protein	-26.6
sgcB	putative enzyme IIB component of PTS	-27.0
ynaJ	putative inner membrane protein, DUF2534 family	-28.1
sstT	sodium:serine/threonine symporter	-28.1
csiD	carbon starvation protein	-28.2
bolA	stationary-phase morphogene, transcriptional repressor for mreB; also regulator for dacA, dacC, and ampC	-28.2
wrbA	NAD(P)H:quinone oxidoreductase	-28.5
srlD	sorbitol-6-phosphate dehydrogenase	-28.6
gabD	succinate-semialdehyde dehydrogenase I, NADP-dependent	-29.3
znuC	zinc transporter subunit: ATP-binding component of ABC superfamily	-30.3
elaB	ribosome-binding protein, probably membrane-anchored, function unknown	-31.2

Gene	Annotation	MG1655 CusR
ycaC	putative hydrolase, isochorismatase family	-31.9
yccJ		-33.6
ygiM	SH3 domain protein	-34.4
malE	maltose transporter subunit	-34.8
ypeA	putative acyltransferase with acyl-CoA N-acyltransferase domain	-35.3
talB	transaldolase B	-35.7
nadE	NAD synthetase, NH3/glutamine-dependent	-35.8
otsB	trehalose-6-phosphate phosphatase, biosynthetic	-36.4
selD	selenophosphate synthase	-37.3
ynhF		-38.1
grxB	glutaredoxin 2 (Grx2)	-38.7
yiaG	putative transcriptional regulator, HTH_CROC1 family	-40.1
mntS	Mn(2)-response protein, MntR-repressed	-41.1
ydjA	putative oxidoreductase	-42.1
ytjA		-42.8
cyoB	cytochrome o ubiquinol oxidase subunit I	-44.1
gltA	citrate synthase	-44.5
glpF	glycerol facilitator	-44.9
cyoE	protoheme IX farnesyltransferase	-45.0
yeaC		-51.3
osmE	DNA-binding transcriptional activator	-54.6
srlA	glucitol/sorbitol-specific enzyme IIC component of PTS	-62.5
srlE	glucitol/sorbitol-specific enzyme IIB component of PTS	-63.0
ompF	outer membrane porin 1a (Ia, b, F)	-64.8
cyoA	cytochrome o ubiquinol oxidase subunit II	-66.9

Table 7.1.13 HK copy number for acceptor photobleaching experiments. Values taken from PhD thesis Erik Sommer [203]

	Copy number	
HK	CFP-fusion	YFP-fusion
AtoS	9000	3800
BaeS	8200	2800
BarA	1400	3000
BasS	4100	7500
CitA	3700	3000
CpxA	2300	3000
CreC	2000	2500
CusS	2000	1160
DcuS	7600	12000
EnvZ	1800	180
HydH	2400	3400
NarQ	11300	9000
NarX	2700	3000
PhoQ	21000	15000
PhoR	2000	3900

QseC	15800	18000
TorS	6500	6000
UhpB	1800	1700
YdpA	1000	1900
YedV	12800	10000
YfhK	2000	1700

7.2 Figures

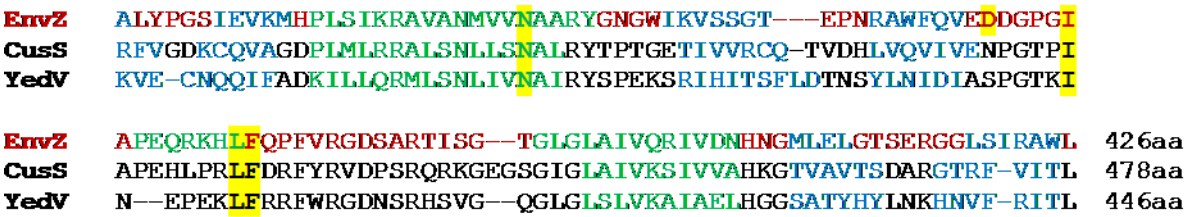

Figure 7.2.1 Sequence alignment of the ATP binding domains of EnvZ, CusS and YedV. Amino acids N347, D373, I378, L386 and F387 (yellow) were described to bind ATP in the histidine kinase EnvZ [71]; colours show the secondary structure of the proteins (blue= ß-strand; green= α-helix)

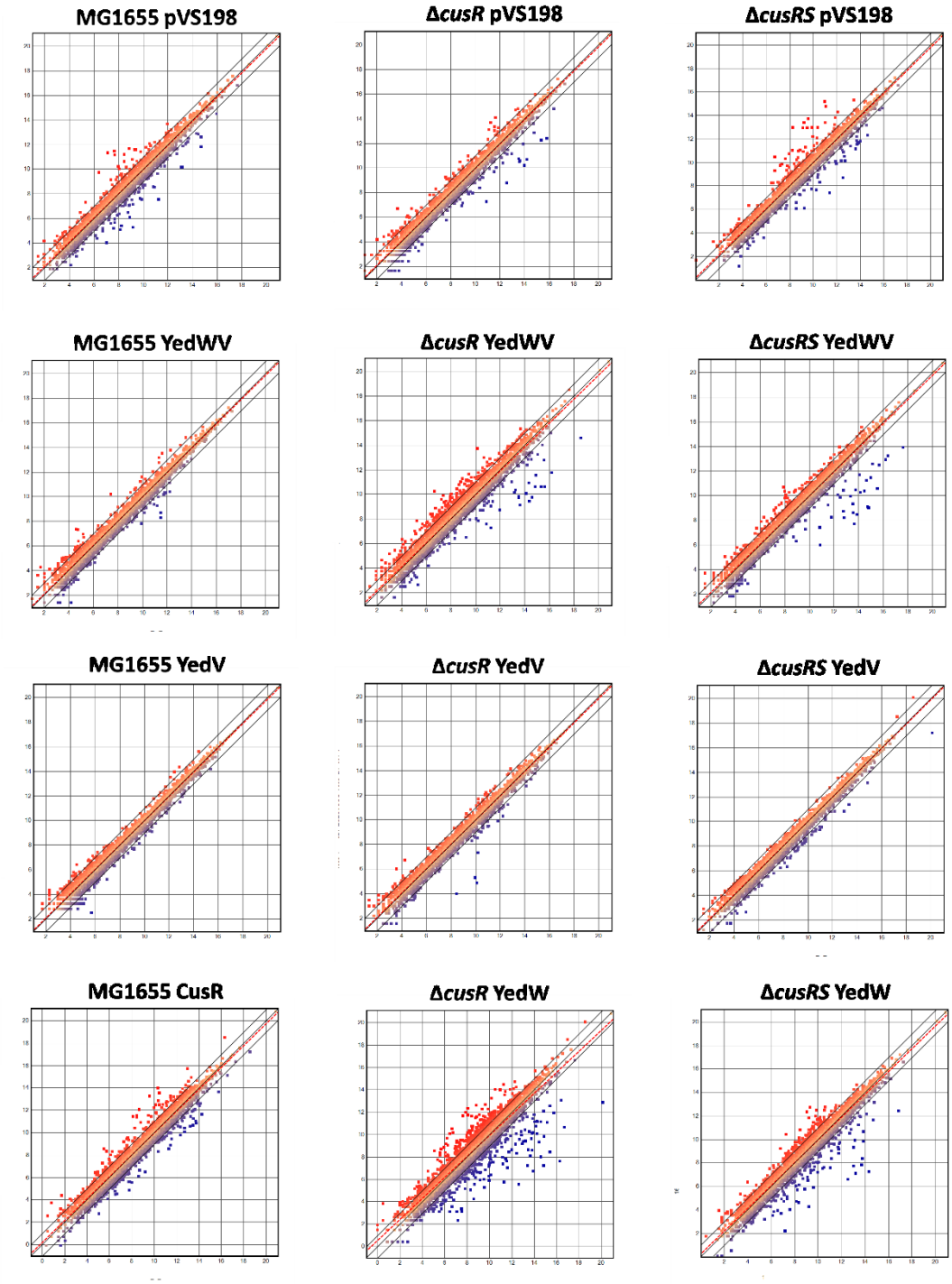

Figure 7.2.2 Scatter plots of RNA sequencing duplicates of all samples with MG1655 background.

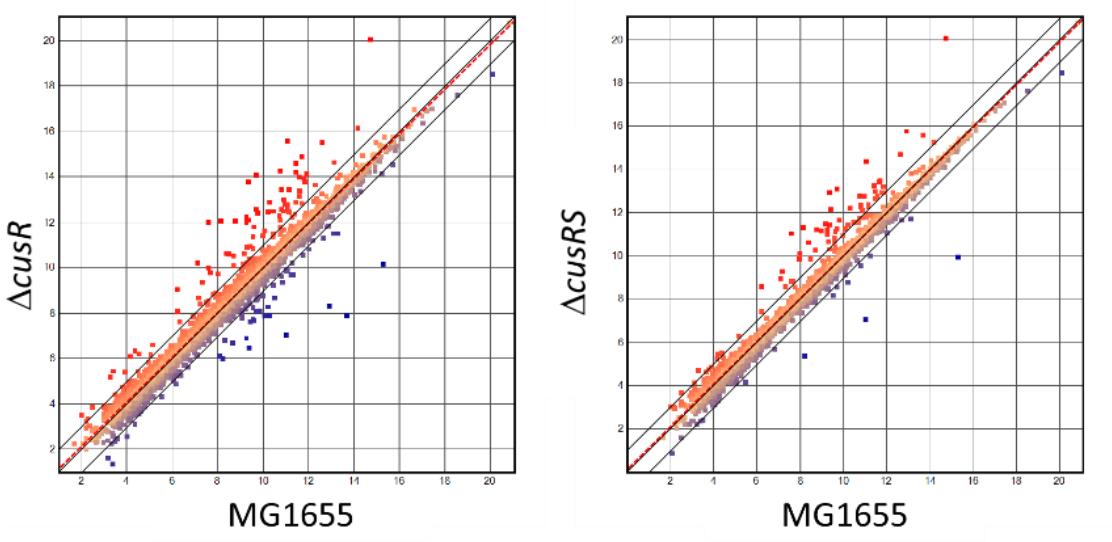

Figure 7.2.3 Scatter plots of MG1655 pVS198→Δ*cusRS*$_{MG}$ pVS198 and MG1655 pVS198→ Δ*cusR*$_{MG}$ pVS198.

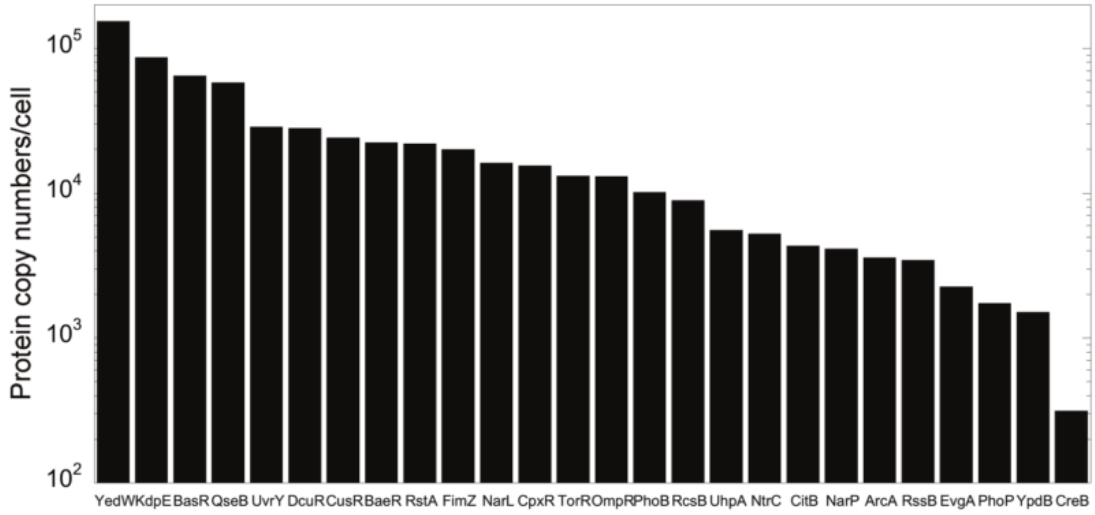

Figure 7.2.4 RR copy numbers for acceptor photobleaching experiments. Figure taken from PhD thesis Erik Sommer [203]

8 List of Figures and Tables

Table 8.1: List of Figures

Table 8.2: List of Tables