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REVIEW

# Rewiring the functional complexity between Crc, Hfq and sRNAs to regulate carbon catabolite repression in *Pseudomonas*

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## Abstract

*Pseudomonas* species are the most versatile of all known bacteria for metabolic flexibility and the extent of host range from plants to humans that remains unmatched. The evolution of diverse metabolic strategies in these species to adapt to the fluctuating environment guarantees high fitness as well as the ability to withstand stress at multiple levels. These abilities in *Pseudomonas* species are imprinted by an adaptable genetic repertoire through the integration of external and internal signals via complex regulatory networks. One of the main regulatory networks that lead to optimal growth, survival and cellular robustness is the phenomenon of carbon catabolite repression (CCR). Even though a large array of information is available, the molecular machinery and the mechanism of CCR in *Pseudomonas* are distinctly diverse from *Escherichia coli* and *Bacillus subtilis.* In *Pseudomonas*, the Crc and Hfq proteins, CbrAB two-component systems and the CrcZ/CrcY small RNA are key components of CCR. The main focus of this review is to elucidate the mechanism of CCR and the accessories involved in regulation of preferred carbon source utilisation over non-preferred ones and how CCR influences the virulence, antibiotic resistance, bioremediation and plant growth promotion pathways. Furthermore, we have also tried to shed some light on the "omics" approaches which can provide deep mechanistic insights into the regulation of CCR. Understanding the mechanistic picture of key regulatory entities and mechanism responsible for metabolic flexibility will create opportunities for exploitation of these versatile prokaryotes in several biotechnological processes.

**Keywords** Catabolite repression control (CCR) · CbrAB · Crc · CrcZ/Y · Hfq · *Pseudomonas*

## Introduction

Bacteria sense environmental changes by perceiving extracellular hints such as the concentration of nutrients for example, carbon, nitrogen, phosphate, iron, sulphur, and growth conditions for instance pH, temperature, oxygen availability, osmotic stress and survive in response to these perturbations (Shimizu 2014). The environmental perturbations send signal to the cells sensing the change which induces the secretion of auto-inducer molecules leading to the sensitisation of whole community. This allows the bacterial community to synchronise gene expression, and thereby carry out collective activities for growth and survival. Induction of changes sensed by individual cells may lead to changes in the whole community, through quorum sensing. The signals provided to the transcriptional regulatory systems shape the physiological and morphological adjustments that facilitate effective adaptation and survival of the whole bacterial community (Seshasayee 2006). This metabolic versatility shapes the ecological fitness of an organism by coordinating a number of distinctive global regulatory networks leading to expression (Shimizu 2014).

In a face-changing environment where numerous carbon sources are available at concentrations that define the survival of microorganisms, bacterial species activate universal regulation systems that synchronise metabolism. The presence of variety of substrates in a natural environment allows bacteria to either co-utilise carbon sources or utilise preferential ones that are most effective for growth. The selection of a preferred carbon source over others and inhibition of the uptake machinery or genes essential for the catabolism of non-preferred ones at the same time is regulated precisely. The regulated selection of preferred carbon source over non-preferred ones is termed as carbon catabolite repression (CCR). Originally CCR was illustrated in *Escherichia coli*

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for utilisation of sugars in a hierarchical manner (Magasanik 1970). CCR can be carried out in two ways: first, inhibition of expression of enzymes of the pathways for non-preferred carbon source and second, reshuffling of metabolism for activation of appropriate genes leading to the assimilation of preferred carbon sources (Rojo 2010). Being a complex mechanism, CCR can be arbitrated by various regulatory systems. The fundamental molecular regulatory networks differ among individual bacterial groups. The underlying machinery and the regulatory partners required for CCR have been studied extensively in *Enterobacteria, Firmicutes* and in *Pseudomonas* (reviewed in Gorke and Stulke 2008).

## CCR in *Pseudomonas*: the general mechanism

*Pseudomonas* is a genus of ubiquitous bacteria found in a wide range of habitats such as soil, water, animals and plant influencing them either positively or negatively. Some well-known examples include opportunistic animal pathogen *P. aeruginosa* (Sonnleitner et al. 2017); plant pathogen *P. syringiae* (Chakravarthy et al. 2017; Filiatrault et al. 2013); bioremediation agent *P. putida* (Basu et al. 2006) and *P. fluorescens* as plant growth promoting and bio-control agent (Liu et al. 2017). *Pseudomonas* is well adapted to various environments as they can utilise a wide array of carbon sources (Dos Santos et al. 2004; Molina et al. 2000) but are reported to utilise a limited number of sugars such as glucose, glucuronic acid and fructose (Daddaoua et al. 2009; Silby et al. 2011). The metabolic patterns in these microbes also govern a lifestyle that acclimatises to environmental conditions where sugars are in limited concentrations. Glucose utilisation machinery is organised in three-tiered metabolic system that generates 6-phosphogluconate and has remarkable differences as compared to *Escherichia coli* and *Bacillus* species. The glucose is transported and metabolised through the phosphoenolpyruvate phosphotransferase system (PTS) in these bacteria whereas in *Pseudomonas*, glucose crosses the outer membrane into the periplasmic space through the OprB-1 porin and is directly transported into the cell or can be oxidised to gluconate or 2-ketogluconate in the periplasmic space. All the metabolites produced during periplasmic glucose oxidation have their own transporters through which they can be transported inside the cell. Once imported inside the cell, glucose, gluconate and 2-ketogluconate are oxidised through the Entner–Doudoroff pathway (Del Castillo et al. 2007). Differences in transport of glucose also influence the regulation of catabolism in *Pseudomonas*. In *E. coli*, the PTS, a multi-protein phosphorylation cascade helps in glucose uptake and metabolism. Whereas in *Pseudomonas,* glucose metabolism is regulated by one-component systems (OCSs) HexR, PtxS, PtxR and GntR transcriptional factors as well as two-component system (TCS) GltR/GtrS transcriptional factor which are induced by intermediates of the ED pathway (Udaondo et al. 2018).

*Escherichia coli* utilises glucose as a preferred carbon source suppressing catabolic pathways of glycerol, organic acids and amino acids by CCR (Postma et al. 1993). On the other hand, metabolism of glucose is under catabolic repression in *Pseudomonas* because organic acids are preferred over carbohydrates. The repression in *Pseudomonas* is reverse of the phenomenon followed in enteric bacteria and it is often referred to as "reverse CCR". For instance, presence of succinate and glucose creates CCR conditions where expression of glucose catabolism enzymes of *P. aeruginosa* was inhibited until succinate was available (Collier et al. 1996). This phenomenon of physiological hierarchy to nutrient accessibility that leads to the preferential utilisation of succinate over other carbon sources for growth is termed as succinate mediated catabolite repression (SMCR). Organic acids succinate and acetate are known to repress enzymes for utilisation of gluconate, glycerol, fructose and mannitol. The sequential hierarchy among amino acid utilisation has also been reported in *P. putida* (Hester et al. 2000a, b; Moreno 2007; Rojo 2010). *P. putida* strain CSV86 has unique ability to utilise aromatic compounds such as naphthalene prior to glucose where succinate does not limit naphthalene degradation but impairs transport system and enzymes for glucose assimilation (Basu et al. 2006, 2007). In presence of succinate and benzoate, benzoate was preferentially metabolised while glucose transport and its metabolism were suppressed in *P. putida* CSV86 (Choudhary et al. 2017).

## The CCR mediators

At present, the regulatory system of CCR in *Pseudomonas* encompasses the following components: Crc and Hfq proteins, the TCS: CbrAB and sRNAs: CrcY/CrcZ. The subsequent sections of the review summarise how these components cooperate and function to execute CCR.

## Crc protein: the global regulator

Catabolite repression control (Crc) protein was originally reported in *P. aeruginosa* during random mutagenesis experiments intended to find catabolite repression relieved mutants of amidase (MacGregor 1991; Wolff 1991). Some *crc* mutants were relieved of repression imparted by succinate over mannitol and glucose transport and enzymes involved in their catabolism. The Crc protein shares up to 25 to 32% sequence homology with DNA repair enzymes of other bacteria but does not show any endonuclease or DNA binding ability (MacGregor 1996). In *P. putida* and

*P. aeruginosa,* Crc regulates induction of *bkd* operons for branched-chain keto acid dehydrogenases. Consequently, in several other *Pseudomonas* species, Crc was described as the regulator of catabolite repression of branched-chain keto acid dehydrogenase (Hester et al. 2000a) enzyme for degradation of alkane (Yuste and Rojo 2001), toluene (Aranda-Olmedo 2005), aromatic compounds (Morales et al. 2004) and biofilm formation (O'Toole et al. 2000). The difference in growth conditions was shown to have differential impact on the expression of *crc* (Ruiz-Manzano et al. 2005). In *P. putida,* Crc binds to *benR* and *alkS* mRNAs and inhibit the translation leading to repression of benzoate and alkane degradation genes respectively (Moreno 2007, 2008). However, the crystal structure of Crc from *P. aeruginosa* lacked nuclease activity (Milojevic et al. 2013). This suggested that there must be some other proteins helping the regulatory action being carried out during CCR. Crc exerts a positive impact on the type III secretion system (T3SS) in *P. aeruginosa* where the master regulator *ExsA* and the Cbr/Crc signalling system regulate T3SS (Dong et al. 2013). Crc is also an up-regulator of rhamnolipid production in *P. aeruginosa* (Yang et al. 2015). Some recent studies have identified Crc as a regulator of muconate production in *P. putida* KT2440 where levels of 4-hydroxybenzoate and vanillate were reduced upon *crc* deletion (Johnson et al. 2017). Virulence of *P. syringae* pv. Tomato DC3000 was influenced by Crc (Chakravarthy et al. 2017) which was also reported to coordinate the overall response to oxidative stress by means of reorganisation of central metabolism in *P. aeruginosa* PAO1 (Corona et al. 2018, 2019).

## Hfq: the "RNA matchmaker"

The Hfq protein was initially discovered as a host factor essential for replication of bacteriophage Qβ in *E. coli* (August et al. 1970). Hfq is a hexameric protein belonging to the class of Sm-family of RNA binding proteins in many bacteria (Zhang et al. 2002) and has a proximal and distal RNA binding site (Sauer and Weichenrieder 2011). Hfq is a regulator in Gram-negative bacteria that assists the regulatory RNA mediated post-transcriptional gene regulation during CCR (Sonnleitner and Blasi 2014; Kambara et al. 2018). During CCR in *P. aeruginosa,* the translation of target transcripts was inhibited directly by Hfq-Crc complex. Crc bound Hfq on its distal part binds to the A-rich motifs on target mRNA near to ribosome binding site (RBS) and regulates the translation (Sonnleitner et al. 2018b).

Being a global regulator, Hfq interacts with sRNAs ranging in size from 37 to 500 nucleotide and stimulate or restrain mRNA translation through positive or negative regulatory mechanisms (Santiago-Frangos and Woodson 2018). The size, conformation and combination of complementary sequences of RNA differ when Hfq mediated regulation is considered. During Hfq-dependent regulation, mRNA and sRNA bind to Hfq on different sites. It has been reported that Hfq binding to the mRNA with or without sRNA influence the fate of mRNAs. For example, during negative regulation, Hfq assists stabilisation and recruitment of sRNA and help to recruit ribonucleases which ultimately degrade mRNAs. In positive regulation sRNAs and Hfq binding stabilises mRNA by changing mRNA folding which allows ribosome access or blocking access of a ribonuclease to protect the mRNA (Kavita et al. 2018). The RNA ligands are highly varied in nature which is reflected in cognate protein partners that comprise of an array of functions related to various enzymes (Butland et al. 2005). Hfq functions as a stabiliser of regulatory sRNAs like CrcZ and CrcX in the absence of their target transcripts by facilitating base-pairing to the mRNAs (Zhang et al. 2002); it helps in the repression of translation and also mRNA activation (Gottesman and Storz 2011; Vogel and Luisi 2011). Thus, the selectivity of substrate for Hfq has to be flexible enough to cooperate with a range of RNA-protein complexes to find a perfect match (Updegrove et al. 2016; Kavita et al. 2018; Santiago-Frangos and Woodson 2018).

The function of Hfq has been established by generation of Hfq deletion strain of *P. aeruginosa* which influences antibiotic susceptibility, energy metabolism, cell wall composition and the levels of c-di-GMP (Sonnleitner et al. 2018b). In *P. aeruginosa* PAO1, Hfq represses three mRNAs namely *amiE* (encoding an aliphatic amidase), *estA* (esterase) and *phzM* (phenazine-specific methyltransferase) (Sonnleitner and Blasi 2014). The translational inhibition applied by Crc operates in association with the Hfq protein to reduce translation of DmpR regulator of the Dmp-pathway (dimethylphenol) for catabolism of phenol in *P. putida* (Wirebrand et al. 2018). Functional characterisation of *Acinetobacter baumannii* lacking Hfq revealed its role in environmental adjustment and virulence by amending stress responses, morphology and virulence factors (Kuo et al. 2017). In *Azotobacter vinelandii,* the gene *gluP* encoding glucose transporter has A-rich Hfq-binding motif and thus glucose transport is under CCR through Crc/Hfq and CbrA/CbrB regulatory systems (Quiroz-Rocha et al. 2017). Hfq protein has a pivotal role as a pleiotropic regulator mediating complex CCR regulation that influences metabolism, virulence, quorum sensing and stress response in *Pseudomonas* and related bacterial species.

## CrcZ, CrcY: the regulatory sRNAs

The role of small RNAs (sRNAs) (~ 50–300 nucleotides) has been determinedly established as important regulators of metabolism in bacteria. The sRNAs are known for

regulating bacterial environmental response pathways and the most comprehensively studied sRNAs are known to regulate mRNAs of target genes by defective and short base pairing as trans-encoded sRNAs. Some sRNAs bind at or near the RBS and block translation by obstructing ribosomes. Additional types of sRNAs bind far-away from the target site and interfere with ribosome binding through either inhibiting formation of secondary structures or altering mRNA stability (Storz et al. 2011). The group of sRNAs that sequester and titrate the RNA binding proteins of CsrA/RsmA family control translation initiation. These small proteins explicitly bind to GGA trinucleotide situated in the 5′ leader sequence of target mRNAs and inhibit their translation.

One such protein-RNA system is the CrcZ/Crc system that works in catabolite repression control in *Pseudomonas* species. In conditions where CCR is operated, Crc protein binds to AC rich motifs within or adjacent to RBS of target mRNAs inhibiting their translation. When CCR is relieved, the regulatory RNA CrcZ, CrcY/CrcZ and CrcZ/CrcX in *P. aeruginosa* PAO1, *P. putida,* and *P. syringae* respectively are projected to bind and trap the protein (Sonnleitner et al. 2009; Moreno et al. 2012; Filiatrault et al. 2013). However, the structural and biochemical studies posed a question against the role of Crc mediated CCR in *P. aeruginosa* PAO1 as purified Crc was neither able to bind the aliphatic amidase encoding *amiE* mRNA (target mRNA) nor to CrcZ sRNA (Milojevic et al. 2013). Numerous genes of *P. aeruginosa* involved in catabolic pathways are known to be inhibited post-transcriptionally by Hfq by binding on the distal face of Hfq during growth on succinate (Sonnleitner et al. 2018b). The Hfq binds to CrcZ and Crc protein which as a complex exert regulation (Rojo 2010; Sonnleitner and Blasi 2014). The response of a bacterium to a particular signal is influenced by the concurrent cellular environment of Hfq-dependent sRNAs, Crc protein, target mRNAs and the capability of these RNAs to compete with each other so that precise regulatory pathways are arranged on a priority basis (Santiago-Frangos and Woodson 2018). To understand the interdependence of Hfq-sRNA mediated control in *P. aeruginosa*, the possibility whether the regulatory RNA CrcZ can interfere with riboregulation mediated by the sRNAs PrrF1-2 was checked. *antR* mRNA encoding a transcriptional activator of the *antABC* operon is required for anthranilate degradation and is known to be controlled by PrrF1-2 sRNA. CrcZ cross-regulates mRNAs of *antR* with Hfq-mediated riboregulation. In iron limitation and growth on preferred carbon source, *antR* translation was repressed by PrrF1-2 and Hfq. In non-CCR conditions, CrcZ competes for Hfq binding which interferes with PrrF1-2 binding, activating *antR* translation and degradation of anthranilate (Sonnleitner et al. 2017).

## CbrA-CbrB: the two-component system

The immense versatility and ability to quickly adapt to the fluctuating nutritional environment is due to the presence of well-regulated two-component systems (TCSs) programmed in genomes of bacteria. The TCSs encompasses a sensor kinase (SK) and a response regulator (RR). The autophosphorylation caused in the histidine kinase (HK) domain of the SK is induced by environmental stimulus leading to subsequent transfer of phosphate to the acceptor domain of RR triggering activation of regulator protein (von Bodman et al. 2008). Thus, activated RR stimulates expression of genes critical for adjustment towards the altered environmental condition. This adaptation of bacterial cells through activation of TCS leads to change in physiological functions aiding utilisation of alternative sources when certain nutrients become limiting. When nitrogen or phosphate is limiting the NtrB-NtrC and the PhoR-PhoB are activated for nitrogen and phosphate assimilation, respectively (Bourret and Silversmith 2010). Generally, the N-terminal periplasmic domain of SK senses nutrients or external stimuli priming the activation of respective genes by the RR. Another TCS known as CbrAB has ability to regulate carbon and nitrogen catabolism in *Pseudomonas* and plays a global regulatory role (Nishijyo et al. 2001; Zhang and Rainey 2008). In CbrAB TCS, CbrA is HK and CbrB is RR that regulates and establishes a healthy carbon/nitrogen balance (Sonnleitner et al. 2009, 2012b; Moreno et al. 2012). CbrB is an NtrC family RR that activates the transcription of sRNAs (Nishijyo et al. 2001; Sonnleitner et al. 2012a). The CbrA/CbrB system has several functions in *Pseudomonas* species such as regulation of swarming motility, biofilm formation, antibiotic and stress resistance (Amador et al. 2010; Yeung et al. 2011). The phenotypic analysis in CbrAB mutants of *P. fluorescens* SBW25 resulted in growth deficiency on various carbohydrates and amino acids (Zhang and Rainey 2008). It was also found that the leucine metabolism in *P. aeruginosa* is also under the influence of CbrB/Crc regulation. Leucine is metabolised by enzymes encoded in the *liuRABCDE* gene cluster, where LiuR is the regulator. In the presence of leucine, LiuD was strongly expressed but repressed in the presence of glucose or succinate. These results indicate that in absence of Crc, LiuD expression was independent of the presence of carbon source, however, its expression was impaired in *cbrB*- mutant (Díaz-Pérez et al. 2018). The mutants of *cbrAB* of opportunistic human pathogen *P. aeruginosa* PAO1 also had growth defects (Nishijyo et al. 2001). CbrAB along with Crc regulates the carbohydrate metabolism as well as the amino acid metabolism.

## The definite mechanism of CCR in *Pseudomonas*

In *Pseudomonas* species, the hierarchy of carbon source utilisation emerges through translational repression mediated by Crc protein. The A-rich sequences near the RBS of the mRNAs are crucial for the repression. Crc does not bind to the target mRNA directly; instead, Hfq allows the binding of the A-rich motifs to its distal face. In presence of a non-preferred carbon source (for example, glucose: when catabolite repression is not necessary), Crc/Hfq repression is relieved by expression of a non-coding RNA-CrcZ or CrcY, bearing various copies of the A-rich motif, which sequester Hfq-Crc complex (Fig. 1a). The presence of non-preferred carbon source in the medium shows a strident increase in the levels of CrcZ/Y leading to sequestration of Crc which allows ribosome binding and translation of the mRNAs for utilisation of non-preferred carbon source (Sonnleitner et al. 2018b).

In presence of a favored carbon source (for example, succinate), the concentration of CrcZ/Y depletes and free Crc possibly remains at a concentration high enough to form complex with Hfq that binds to the target mRNAs holding back their translation (Fig. 1b) and thus inhibiting utilisation of non-preferred carbon sources. The level of CrcZ is regulated by CbrAB which is activated to set the hierarchy of carbon source utilisation. Inactivation of CbrB does not show any influence on CrcY expression as CrcY activation relies largely on systems different from CbrAB. Figure 1 illustrates the mechanism of CCR in *Pseudomonas* species and involvement of its mediators- Crc and Hfq proteins, CrcZ/Y and CbrAB. The physiological traits that are governed by

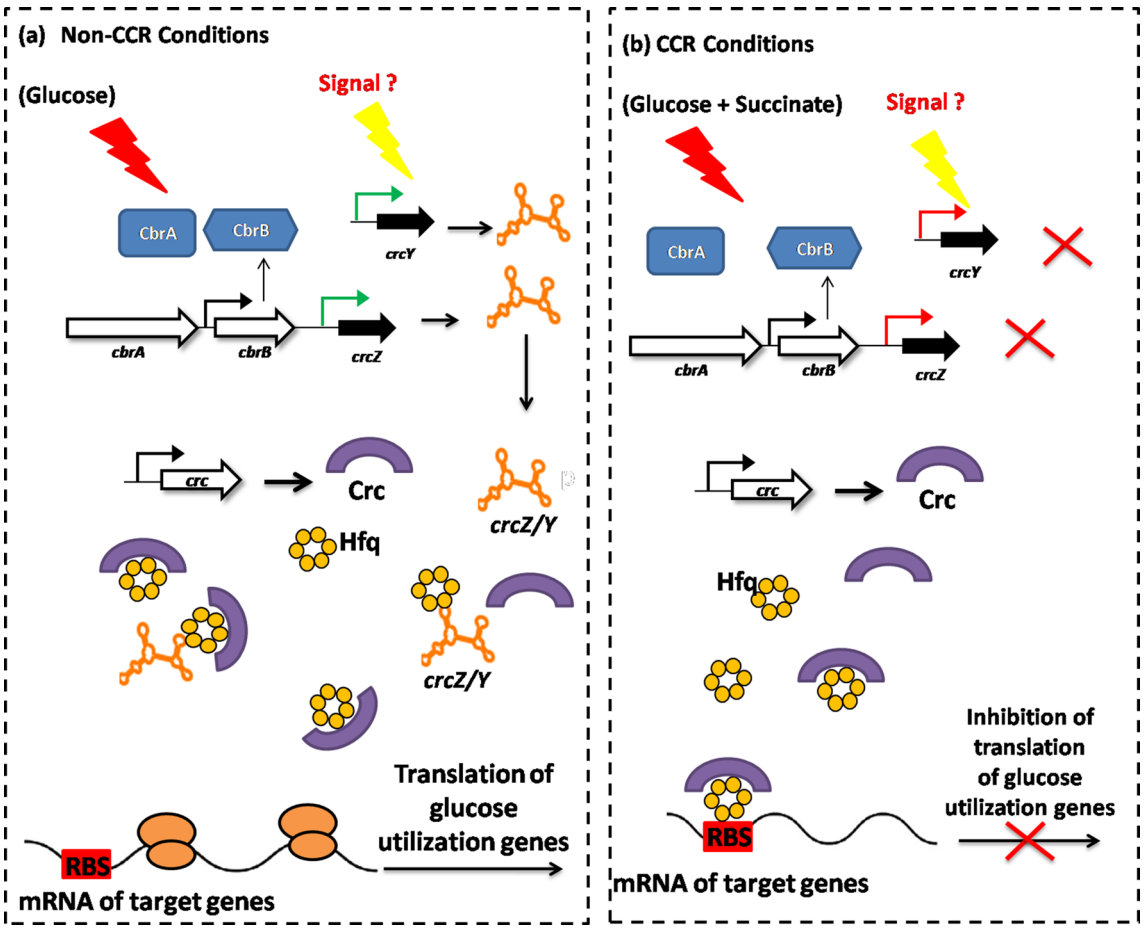


**Fig. 1** Mechanism of succinate mediated catabolite repression (SMCR) in *Pseudomonas*. **a** In the non-CCR conditions (e.g. in presence glucose), Crc binds to Hfq bound sRNA (CrcZ/CrcY) allowing translation of glucose utilisation genes. **b** Under the CCR conditions (SMCR), when preferred and non-preferred carbon source (succinate and glucose respectively) are both present, the Crc-Hfq complex binds to the RBS inhibiting translation of glucose utilisation genes. The repression is relived (under non-CCR conditions) via sequestering of Crc and Hfq by CrcZ whose expression is activated by CbrAB two-component system and CrcY whose expression is controlled by unknown signals. This model is based on the results of the earlier studies (Kavita et al. 2018; Rojo 2010; Sonnleitner and Blasi 2014; Sonnleitner et al. 2018a, b). A green or red arrow indicates activation or inhibition respectively

Crc, Hfq CrcZ/Y and CbrAB in various bacterial species are listed in Table 1.

## Influence of CCR on catabolic pathways of *Pseudomonas*

The activation of a catabolic pathway depends on the combined activity of several global regulators in response to external or internal signals generated due to the cell's need to adapt and survive. The CCR interferes with the stimulation of certain catabolic pathways by employing mediators for down-regulation of particular transcriptional regulators or by interfering with the ability of the regulators to control transcription. Rojo (2010) has extensively reviewed influence of CCR on the degradation of alkane, toluene/xylene and phenol degradation pathways encoded by OCT, Tol and pVI150 plasmids respectively in *P. putida*.

## Influence of CCR on virulence

In *P. aeruginosa* which is a well known human pathogen, expression of virulence genes is governed by CCR. A pathogenic bacterium infects the host, with the prime aim of acquiring nutrients to survive in the environment. Therefore it looks for alternative carbon sources and therefore the metabolism is influenced by CCR. Various virulence systems are known to amend the pathogenicity of *P. aeruginosa.* One of the important mechanisms for virulence regulation is quorum sensing (QS) which employs secretion of small diffusible signal molecules in surrounding environment in order to synchronise gene expression and concerted behaviour, for example biofilm formation (Miller and Bassler 2001). It has been reported that the *Pseudomonas* quinolone signalling (*pqs*) is affected by Crc in a nutrient-dependent manner (Zhang et al. 2014). The

**Table 1** Crc, Hfq and sRNA mediated regulation in various bacterial species

| Bacteria | Physiological trait/mechanism | Global regulators (proteins/sRNAs) | References |
|---|---|---|---|
| *Pseudomonas aerugenosa* | Swimming, swarming and twitching motility, bio film formation and EPS production | Crc | O'Toole et al. (2000) |
| *Pseudomonas putida* | Complex branched-chain keto acid dehydrogenase | Crc | Hester et al. (2000a) |
| *Pseudomonas putida* *Pseudomonas aeruginosa* | Branched-chain keto acid dehydrogenase (BCKAD), glucose-5-phosphate dehydrogenase, and amidase | Crc | Hester et al. (2000b) |
| *Pseudomonas putida* GPo1 | Alkane Degradation | Crc | Yuste and Rojo (2001) |
| *Pseudomonas putida* | Alkane degradation | Crc | Ruiz-Manzano et al. (2005) |
| *Pseudomonas fluorescens* SBW25 | Histidine utilisation | CbrAB and NtrBC | Zhang and Rainey (2007), Zhang and Rainey (2008) |
| *Acinetobacter baylyi* | Protocatechuate degradation | Crc | Zimmermann et al. (2009) |
| *Vibrio cholera* | Virulence | Hfq | Vincent et al. (2012) |
| *Pseudomonas syringae* pv. *tomato* DC3000 | Virulence | Crc, CrcZ, CrcX | Chakravarthy et al. (2017), Filiatrault et al. (2013) |
| *Pseudomonas aeruginosa* | Type III Secretion System | Crc | Dong et al. (2013) |
| *Pseudomonas aeruginosa* and *Pseudomonas Putida* | Hierarchical management of carbon sources | CbrA/B systems | Valentini et al. (2014) |
| *Pseudomonas putida* KT2440 | Conversion of aromatic lignin monomers to muconate | Crc | Johnson et al. (2017) |
| *Acinetobacter baumannii* | Biofilm formation, airway epithelial cell adhesion and invasion, survival in macrophage | Hfq | Kuo et al. (2017) |
| *Pseudomonas putida* KT2440 | Enhanced muconate production | Crc | Johnson et al. (2017) |
| *Azotobacter Vinelandii* | Glucose uptake through GluP transporter | CbrA/CbrB and Hfq-Crc systems | Quiroz-Rocha et al. (2017) |
| *Pseudomonas fluorescens* SBW25 | Xylose utilisation | Hfq/Crc/sRNA and CbrAB | Liu et al. (2017) |
| *Pseudomonas aeruginosa* | Post-transcriptional regulation during carbon catabolite repression | Hfq/Crc/sRNA complexes | Sonnleitner et al. (2018b) |
| *Pseudomonas putida* | Iron homeostasis | Hfq/Crc/sRNA | Sánchez-Hevia et al. (2018) |

RhlAB system is known for rhamnolipid synthesis which is a virulence factor required during *P. aeruginosa* lung infection. Several proteases and chaperones together serve to maintain quality control of cellular proteins that are known as the protein quality control (PQC) systems. The PQC of Gram-negative bacteria has Lon and ClpX (clp, caseinolytic protease) which are ATP-dependent proteases (Mogk and Bukau 2006). Crc induces Rhl production by down-modulation of Lon protease. The *hfq* and *crc* deletion leads to reduced production of the QS signal molecule N-Butyryl-homoserine lactone (C4-HSL) in *P. aeruginosa* (Sonnleitner et al. 2006; Yang et al. 2015). The *rhl* QS signal C4-HSL production was balanced by disruption of Lon protease which is influenced by c*rc* deletion. It was also reported that the repression of *rhl* QS by ClpX was Lon independent. In addition, ClpP protease is a negative regulator of *rhl* QS therefore, ClpPX in combination have a role in linking CCR and PQC mediated regulation in *P. aeruginosa* (Yang and Lan 2016).

## Influence of CCR on antibiotic resistance

Apart from virulence, CCR also plays an important role in the development of antibiotic resistance. A steady rise in drug resistance among the clinical isolates poses a challenge towards treatment of various infectious diseases. It becomes important to understand the molecular mechanism of drug resistance in depth to enhance sensitivity of bacteria to the antibiotics in use. In *P. aeruginosa* antibiotics, amino-acids and carbon sources share same ports of entry for getting inside the cell and the transporter encoding genes are frequently controlled by CCR. In *P. aeruginosa* defects in type III secretion and motility due to Crc deletion were reported. The mutant strain became more susceptible to antibiotics such as beta-lactams, aminoglycosides, fosfomycin and rifampicin (Linares et al. 2010). The *hfq* deletion in *P. aeruginosa* strains also lead to increased susceptibility towards various antibiotics. During CCR i.e. by adding non-preferred carbon source, the expression of CrcZ increased sequestering Hfq which lead to enhanced antibiotic sensitivity (Sonnleitner et al. 2018a). Thus, CCR mediators can be great targets for generating ways for making *P. aeruginosa* more sensitive to different classes of antibiotics.

## Influence of CCR on bioremediation pathways

The molecular means of CCR are important in understanding the behaviour of bacterial species in the environment where they degrade xenobiotic compounds. These compounds are the ones whose concentrations are built up as they are difficult to degrade and create environmental pollution. A number of strains of *Pseudomonas* had been reported for bioremediation potential in laboratory condition (reviewed in Das and Chandran 2011). However, degradation of any of these compounds may be influenced by the presence of multiple carbon sources in natural environment. Contamination by aromatic compounds poses environmental problems with delayed degradation as they are not the preferred carbon compounds for majority of bacteria. It has been reported that succinate being a preferred carbon source, represses degradation of benzoate and alkanes in laboratory conditions, where the global regulator Crc has a role to play (Wang and Shao 2013). Crc is known to be involved in CCR caused by succinate that leads to repression of a number of genes involved in sugar metabolism in both *P. aeruginosa* (Collier et al. 1996; MacGregor 1996) and *P. putida* (Hester et al. 2000a, b). Crc modulates alkane degradation pathway encoded in the OCT plasmid of *P. putida* with varying levels of Crc in accordance with the growth conditions (Ruiz-Manzano et al. 2005). When *P. putida* was grown in association with plant roots, the root exudates reduced phenanthrene degradation (Rentz et al. 2004). This is suggestive of an important fact that CCR may be well operational in natural environments where concentrations of carbon sources are often limiting leading to hierarchical utilisation or repression of certain carbon sources. An interesting approach was suggested wherein the influence of trace amounts of preferred substrate was checked. Use of low concentrations of succinate in *P. putida* mt-2 batch culture improved the toluene removal efficiency (Tsipa et al. 2017). Similarly, strategies for bioremediation of other xenobiotics compounds can be optimised using an in-depth understanding of the molecular networks of CCR and mediators to generate a superior bioremediation agent.

## Influence of CCR on plant growth promotion pathways

Microbial strains present in the rhizosphere that can improve growth of plants are known as plant growth promoting rhizobacteria (PGPR) (Kloepper et al. 1980). The PGPR have been explored as bio-inoculants in the fields for several decades. It would be highly desirable to develop multi-trait PGPR strains that can fix nitrogen, solubilise phosphate, control disease and have other plant beneficial traits. Several PGPR strains are routinely employed but their positive responses to plants are often limited to laboratory or greenhouse and the results are inconsistent in field conditions. The plant roots secrete organic compounds in root exudates that help microbes for survival in the rhizosphere and they in return promote plant growth. The compositions of root exudates vary according to the plant species and often comprise

complex mixtures. Presence of various organic acids and sugars in rhizosphere execute CCR for preferential utilisation of carbon sources. This facilitates the bacterium to compete and survive among existing microbial communities. While it is not yet clear how bacteria choose the substrate in a rhizosphere where CCR could be regulating metabolism, various experiments have been conducted in vitro with commonly found organic acids present in the root exudates. For instance, succinate and malate were reported to repress the gluconate production which was shown to be the main mechanism of mineral phosphate solubilisation (MPS) in plant growth promoting fluorescent *Pseudomonas* (Patel et al. 2011). Similarly, in our laboratory, the biochemical basis of SMCR of MPS in two strains of *Klebsiella* (SM6 and SM11) was established. The SMCR was operative on glyoxylate shunt enzymes (isocitrate lyase and glyoxylate oxidase) hence leading to repression of oxalate mediated MPS (Rajput et al. 2013). Furthermore, it was found that *iclR* repressor of *aceBAK* operon was the key regulator of the MPS repression. Therefore, generation of *iclR* null mutants in both the strains relieved repression of MPS up to 54% and 59% and improved PGP activities as compared to that of wild type strains even in presence of succinate (Rajput et al. 2015). A similar type of biochemical and molecular basis of SMCR is yet to be established in PGP *Pseudomonas* species. A strategy of similar kind may be employed when phosphate solubilisation or other PGP traits are influenced by the components of root exudates and CCR. By these means repression relieved strains can be developed which may further improve plant growth under natural soil conditions also. A more comprehensive genome mining effort need to be carried out to unravel the genetic basis underlying the CCR in *Pseudomonas* and other rhizobacterial species which would be critical for generation of efficient biofertilisation strategies.

## Promising genome-wide methodologies for rewiring the functional complexity between Crc, Hfq and sRNAs

The regulated expression of common responses in bacteria is a consequence of simultaneous integration of multiple signals conferring plasticity, versatility and efficiency. To explore the contribution of each and every input to the expression machinery, it is necessary to analyse the post-transcriptional regulation in *Pseudomonas* species. The Crc protein, Hfq and sRNAs are the main players of the post-transcriptional regulation. To understand the interlaced regulation, a combination of many omics approaches may produce a broad representation of bacterial adaptation to the external milieu.

The potential roles of Crc, Hfq and sRNAs in different bacteria have been characterised by phenomic, transcriptomic and proteomic analyses of knockouts of respective genes. Role of Hfq was established in *P. aeruginosa* (Sonnleitner et al. 2006), *Sinorhizobium meliloti* (Torres-Quesada et al. 2010), *Brucella melitensis* (Cui et al. 2013), *Serratia* sp. ATCC 39006 (Wilf et al. 2013), *E. coli* (Bilusic et al. 2014), *Clostridium difficile* (Boudry et al. 2014), *Yersinia pestis* (Deng et al. 2014), *Bacillus subtilis* (Hämmerle et al. 2014) by using omics approaches.

The transcriptome data revealed the influence of Hfq on genes involved in bacterial metabolism suggesting role of Hfq in controlling the metabolic versatility of *P. fluorescens*. The ribosome profiling experiments were performed to dissect the role of Hfq in regulation at the levels of transcript abundance and translation. The data revealed the negative role of Hfq by controlling translation of mRNAs encoding transport system and enzyme-based systems for amino acid and carbohydrate metabolism, siderophore utilisation, secondary metabolite secretary pathway (Type II and III) and chemotaxis-related genes. The soluble proteome of *Δhfq* revealed up-regulation of a group of putative lipoproteins by Hfq (Grenga et al. 2017). The role of lipoproteins has been reported in amino acid and carbohydrate metabolism, siderophore utilisation, secondary metabolite secretary and chemotaxis related genes in *P. aeruginosa*. A genome wide survey predicted lipoprotein PA0953 as thioredoxin, PA2993 and PA1048 to be involved in thiamine biosynthesis and type VI secretion respectively, which is suggestive of the role of lipoprotein in amino acid metabolism, pathogenesis, etc. Furthermore, it was reported that the outer-membrane lipoproteins belonging to the OprM, OmpR, OmpJ encode antibiotic efflux systems; OmpQ for pyoverdine recycling OpmD for quorum sensing (Remans et al. 2010).

To understand the global post-transcriptional effect of Crc on the physiology of *P. aeruginosa*, transcriptome and proteome of a Crc deficient mutant were analysed against the wild type strain using a post-transcriptional variation (PTV) approach. This study presented a comprehensive map of the Crc post-transcriptional regulon and the mechanism of hierarchical assimilation of carbon sources where Crc played a key role in keeping bacterial homeostasis and consequently metabolic robustness. In addition, the results indicated that CCR in *P. aeruginosa* is also involved in the regulation of other elements of bacterial physiology such as iron uptake genes, siderophore biosynthesis and uptake, xenosiderophore uptake and heme uptake, although the effect of this regulation on bacterial iron homeostasis has not been explored in detail (Corona et al. 2018).

Many functional studies have shown the mutual cooperation of global posttranscriptional regulators such as Crc and Hfq in controlling the destiny of targeted transcripts. A major loophole in existing understanding has been the lack

of structural considerations for cooperation between these global regulators for tight regulation of cellular processes. Pei et al (2018) reported the structural details of Crc using high-resolution cryo-EM structures explaining how Crc can support Hfq in direct translational repression when bound to a translational initiation region on targeted transcripts by forming a multi-component assembly.

RNAseq based transcriptome analysis was carried out with strains of *P. aeruginosa* PAO1 (wild type), PAO1*hfqΔ* and PAO1*Δcrc*. It was found that Hfq-dependent translational regulation was carried out through Crc, Hfq and RNA interaction. This investigation revealed a considerable overlapping interplay between the Crc and Hfq regulon along with protein–protein interaction data supporting rigorous activity between Crc and Hfq protein. Furthermore, the pull-down assays and protein–protein and protein-RNA interactions found that the sRNA binds to the distal surface of Hfq bound Crc complex. In addition to these findings, biochemical and biophysical studies suggest that Crc and Hfq assemble in the presence of RNAs with A-rich motifs (Sonnleitner et al. 2018b). These results were in agreement with the observation of Moreno et al. (2015). The strong interaction between Crc and Hfq enhances the stability of Hfq/Crc/RNA complexes facilitating Hfq-mediated translational repression. Moreover, Crc also interferes with regulatory RNA to Hfq complex formation and thus has a role in riboregulation. In *P. aeruginosa hfq* deletion strain, it had increased susceptibility to different classes of antibiotics. The transcriptome analyses pointed out the impact of Hfq on mechanisms such as import and efflux, energy metabolism, cell wall composition as well as on the c-di-GMP levels in antibiotic susceptibility. These studies hence have helped in understanding the role of CrcZ in sequestration of Hfq that ultimately enhances the sensitivity to antibiotics (Sonnleitner et al. 2018a).

## Conclusion

CCR allows optimum metabolism that is also energetically favorable to achieve efficient growth and enhance the competitiveness of bacteria in their natural habitat. The Crc, Hfq and sRNAs regulate the gene expression in an environmental milieu which has implications in the degradation of various xenobiotic compounds. Complete understanding of the underlying molecular mechanisms behind CCR regulatory networks can help in optimising agricultural, environmental and industrial applications, designing tailor-made biocatalysts and in understanding plant-pathogen or animal-pathogen interactions. The advanced high throughput sequencing and bioinformatics combined with novel approaches including quantitative proteomics, RNAseq and other omics techniques can present a significant breakthrough in discovering and defining exciting mechanisms of regulatory networks.

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## Compliance with ethical standards

**Conflict of interest** No potential conflict of interest was reported by the authors.

## References

Amador CI, Canosa I, Govantes F, Santero E (2010) Lack of CbrB in *Pseudomonas putida* affects not only amino acids metabolism but also different stress responses and biofilm development. Environ Microbiol 12:1748–1761. https://doi.org/10.1111/j.1462-2920.2010.02254.x

Aranda-Olmedo I, Ramos JL, Marqués S (2005) Integration of signals through Crc and PtsN in catabolite repression of *Pseudomonas putida* TOL plasmid pWW0. Appl Environ Microbiol 71:4191–4198. https://doi.org/10.1128/AEM.71.8.4191-4198

August JT, Eoyang L, De Fernandez MT et al (1970) Phage-specific and host proteins in the replication of bacteriophage. RNA Feder Proc 29:1170–1175

Basu A, Apte SK, Phale PS (2006) Preferential utilization of aromatic compounds over glucose by *Pseudomonas putida* CSV86. Appl Environ Microbiol 72:2226–2230. https://doi.org/10.1128/aem.72.3.2226-2230.2006

Basu A, Shrivastava R, Basu B, Apte SK, Phale PS (2007) Modulation of glucose transport causes preferential utilization of aromatic compounds in *Pseudomonas putida* CSV86. J Bacteriol 189:7556–7562. https://doi.org/10.1128/JB.01235-07

Bilusic I, Popitsch N, Rescheneder P et al (2014) Revisiting the coding potential of the *E. coli* genome through Hfq co-immunoprecipitation. RNA Biol 11:641–654. https://doi.org/10.4161/rna.29299

Boudry P, Gracia C, Monot M et al (2014) Pleiotropic role of the RNA chaperone protein Hfq in the human pathogen *Clostridium difficile*. J Bacteriol 196:3234–3248. https://doi.org/10.1128/JB.01923-14

Bourret RB, Silversmith RE (2010) Two-component signal transduction. Curr Opin Microbiol 13:113–115. https://doi.org/10.1016/j.mib.2010.02.003

Butland G, Peregrín-Alvarez JM, Li J et al (2005) Interaction network containing conserved and essential protein complexes in *Escherichia coli*. Nature 433:531–537. https://doi.org/10.1038/nature03239

Chakravarthy S, Butcher BG, Liu Y et al (2017) Virulence of *Pseudomonas syringae* pv. tomato DC3000 is influenced by the catabolite repression control protein Crc. Mol Plant Microbe Interact 30:283–294. https://doi.org/10.1094/MPMI-09-16-0196-R

Choudhary A, Phale PS, Purohit H (2017) Benzoate transport in *Pseudomonas putida* CSV86. FEMS Microbiol Lett. https://doi.org/10.1093/femsle/fnx118

Collier DN, Hager PW, Phibbs PV Jr (1996) Catabolite repression control in the *Pseudomonads*. Res Microbiol 147:551–561. https://doi.org/10.1016/0923-2508(96)84011-3

Corona F, Reales-Calderón JA, Gil C et al (2018) The development of a new parameter for tracking post-transcriptional regulation allows the detailed map of the *Pseudomonas aeruginosa* Crc regulon. Sci Rep 8:16793. https://doi.org/10.1038/s41598-018-34741-9

Corona F, Martínez JL, Nikel PI (2019) The global regulator Crc orchestrates the metabolic robustness underlying oxidative stress resistance in *Pseudomonas aeruginosa*. Environ Microbiol 21:898–912. https://doi.org/10.1111/1462-2920.14471

Cui M, Wang T, Xu J et al (2013) Impact of Hfq on global gene expression and intracellular survival in *Brucella melitensis*. PLoS ONE 8:e71933. https://doi.org/10.1371/journal.pone.0071933

Daddaoua A, Krell T, Ramos JL (2009) Regulation of glucose metabolism in *Pseudomonas*: the phosphorylative branch and Entner-Doudoroff enzymes are regulated by a repressor containing a sugar isomerise domain. J Biol Chem 284:21360–21368. https://doi.org/10.1074/jbc.M109.014555

Das N, Chandran P (2011) Microbial Degradation of petroleum hydrocarbon contaminants: an overview. Biotechnol Res Int. https://doi.org/10.4061/2011/941810

Del Castillo T, Ramos JL, Rodríguez-Hervá JJ et al (2007) Convergent peripheral pathways catalyze initial glucose catabolism in *Pseudomonas putida*: genomic and flux analysis. J Bacteriol 189:5142–5152. https://doi.org/10.1128/JB.00203-07

Deng Z, Liu Z, Bi Y et al (2014) Rapid degradation of Hfq-free RyhB in *Yersinia pestis* by PNPase independent of putative ribonucleolytic complexes. BioMed Res Int. https://doi.org/10.1155/2014/798918

Díaz-Pérez AL, Núñez C, Meza Carmen V et al (2018) The expression of the genes involved in leucine catabolism of *Pseudomonas aeruginosa* is controlled by the transcriptional regulator LiuR and by the CbrAB/Crc system. Res Microbiol 169(6):324–334. https://doi.org/10.1016/j.resmic.2018.05.004

Dong YH, Zhang XF, Zhang LH (2013) The global regulator Crc plays a multifaceted role in modulation of type III secretion system in *Pseudomonas aeruginosa*. Microbiologyopen 2:161–172. https://doi.org/10.1002/mbo3.54

Dos Santos VM, Heim S, Moore E et al (2004) Insights into the genomic basis of niche specificity of *Pseudomonas putida* KT2440. Environ Microbiol 6:1264–1286. https://doi.org/10.1111/j.1462-2920.2004.00734.x

Filiatrault MJ, Stodghill PV, Wilson J et al (2013) CrcZ and CrcX regulate carbon source utilization in *Pseudomonas syringae* pathovar tomato strain DC3000. RNA Biol 10:245–255. https://doi.org/10.4161/rna.23019

Gorke B, Stulke J (2008) Carbon catabolite repression in bacteria: many ways to make the most out of nutrients. Nat Rev Microbiol 6:613–624. https://doi.org/10.1038/nrmicro1932

Gottesman S, Storz G (2011) Bacterial small RNA regulators: versatile roles and rapidly evolving variations. Cold Spring Harb Perspect Biol. https://doi.org/10.1101/cshperspect.a003798

Grenga L, Chandra G, Saalbach G et al (2017) Analyzing the complex regulatory landscape of hfq—an integrative, multi-omics approach. Front Microbiol 8:1784. https://doi.org/10.3389/fmicb.2017.01784

Hämmerle H, Amman F, Večerek B et al (2014) Impact of Hfq on the *Bacillus subtilis* transcriptome. PLoS ONE 9:e98661. https://doi.org/10.1371/journal.pone.0098661

Hester KL, Lehman J, Najar F et al (2000a) Crc is involved in catabolite repression control of the bkd operons of *Pseudomonas putida* and *Pseudomonas aeruginosa*. J Bacteriol 182:1144–1149. https://doi.org/10.1128/jb.182.4.1144-1149.2000

Hester KL, Madhusudhan KT, Sokatch JR (2000b) Catabolite repression control by Crc in 2xYT medium is mediated by posttranscriptional regulation of bkdR expression in *Pseudomonas putida*. J Bacteriol 182:1150–1153. https://doi.org/10.1128/jb.182.4.1150-1153.2000

Johnson CW, Abraham PE, Linger JG et al (2017) Eliminating a global regulator of carbon catabolite repression enhances the conversion of aromatic lignin monomers to muconate in *Pseudomonas putida* KT2440. Metab Eng Commun 5:19–25. https://doi.org/10.1016/j.meteno.2017.05.002

Kambara TK, Ramsey KM, Dove SL (2018) Pervasive targeting of nascent transcripts by Hfq. Cell Rep 23:1543–1552. https://doi.org/10.1016/j.celrep.2018.03.134

Kavita K, de Mets F, Gottesman S (2018) New aspects of RNA-based regulation by Hfq and its partner sRNAs. Curr Opin Microbiol 42:53–61. https://doi.org/10.1016/j.mib.2017.10.014

Kloepper JW, Leong J, Teintze M et al (1980) Enhancing plant growth by siderophores produced by plant growth-promoting rhizobacteria. Nature 286:885–886. https://doi.org/10.1038/286885a0

Kuo HY, Chao HH, Liao PC et al (2017) Functional characterization of *Acinetobacter baumannii* lacking the RNA chaperone Hfq. Front Microbiol 8:2068. https://doi.org/10.3389/fmicb.2017.02068

Linares JF, Moreno R, Fajardo A et al (2010) The global regulator Crc modulates metabolism, susceptibility to antibiotics and virulence in *Pseudomonas aeruginosa*. Environ Microbiol 12:3196–3212. https://doi.org/10.1111/j.1462-2920.2010.02292.x

Liu Y, Gokhale CS, Rainey PB et al (2017) Unravelling the complexity and redundancy of carbon catabolic repression in *Pseudomonas fluorescens* SBW25. Mol Microbiol 105:589–605. https://doi.org/10.1111/mmi.13720

MacGregor CH, Wolff JA, Arora SK et al (1991) Cloning of a catabolite repression control (crc) gene from *Pseudomonas aeruginosa*, expression of the gene in *Escherichia coli*, and identification of the gene product in *Pseudomonas aeruginosa*. J Bacteriol 173:7204–7212. https://doi.org/10.1128/jb.173.22.7204-7212.1991

MacGregor CH, Arora SK, Hager PW et al (1996) The nucleotide sequence of the *Pseudomonas aeruginosa pyrE-crc-rph* region and the purification of the *crc* gene product. J Bacteriol 178:5627–5635. https://doi.org/10.1128/jb.178.19.5627-5635.1996

Magasanik B (1970) Chapter IX: Glucose effects: inducer exclusion and repression. Cold Spring Harb Monogr Arch 1:189–219. https://doi.org/10.1101/087969100.1.189

Milojevic T, Grishkovskaya I, Sonnleitner E et al (2013) The *Pseudomonas aeruginosa* catabolite repression control protein Crc is devoid of RNA binding activity. PLoS ONE 8:e64609. https://doi.org/10.1371/journal.pone.0064609

Miller MB, Bassler BL (2001) Quorum sensing in bacteria. Annu Rev Microbiol 55(1):165–199. https://doi.org/10.1146/annurev.micro.55.1.165

Mogk A, Bukau B (2006) Protein quality control systems: mechanisms and applications. Microb Cell Fact 5:S8. https://doi.org/10.1186/1475-2859-5-S1-S8

Molina L, Ramos C, Duque E et al (2000) Survival of *Pseudomonas putida* KT2440 in soil and in the rhizosphere of plants under green house and environmental conditions. Soil Biol Biochem 32:315–321. https://doi.org/10.1016/S0038-0717(99)00156-X

Morales G, Linares JF, Beloso A et al (2004) The *Pseudomonas putida* Crc global regulator controls the expression of genes from several chromosomal catabolic pathways for aromatic compounds. J Bacteriol 186:1337–1344. https://doi.org/10.1128/jb.186.5.1337-1344.2004

Moreno R, Rojo F (2008) The target for the *Pseudomonas putida* Crc global regulator in the benzoate degradation pathway is the BenR transcriptional regulator. J Bacteriol 190:1539–1545. https://doi.org/10.1128/JB.01604-07

Moreno R, Ruiz-Manzano A, Yuste L et al (2007) The *Pseudomonas putida* Crc global regulator is an RNA binding protein that inhibits translation of the AlkS transcriptional regulator. Mol Microbiol 64:665–675. https://doi.org/10.1111/j.1365-2958.2007.05685.x

Moreno R, Fonseca P, Rojo F (2012) Two small RNAs, CrcY and CrcZ, act in concert to sequester the Crc global regulator in *Pseudomonas putida*, modulating catabolite repression. Mol Microbiol 83:24–40. https://doi.org/10.1111/j.1365-2958.2011.07912.x

Moreno R, Hernández-Arranz S, La Rosa R et al (2015) The Crc and Hfq proteins of *Pseudomonas putida* co-operate in catabolite repression and formation of RNA complexes with specific target motifs. Environ Microbiol 17(1):105–118. https://doi.org/10.1111/1462-2920.12499

Nishijyo T, Haas D, Itoh Y (2001) The CbrA-CbrB two-component regulatory system controls the utilization of multiple carbon and nitrogen sources in *Pseudomonas aeruginosa*. Mol Microbiol 40:917–931. https://doi.org/10.1046/j.1365-2958.2001.02435.x

O'Toole GA, Gibbs KA, Hager PW et al (2000) The global carbon metabolism regulator Crc is a component of a signal transduction pathway required for biofilm development by *Pseudomonas aeruginosa*. J Bacteriol 182:425–431. https://doi.org/10.1128/JB.182.2.425-431.2000

Patel DK, Murawala P, Archana G et al (2011) Repression of mineral phosphate solubilizing phenotype in the presence of weak organic acids in plant growth promoting fluorescent pseudomonads. Biores Technol 102:3055–306140. https://doi.org/10.1016/j.biortech.2010.10.041

Pei XY, Dendooven T, Sonnleitner E et al (2018) Architectural principles for Hfq/Crc-mediated regulation of gene expression. Elife 8:e43158. https://doi.org/10.7554/eLife.43158

Postma PW, Lengeler JW, Jacobson GR (1993) Phosphoenolpyruvate :carbohydrate phosphotransferase systems of bacteria. Microbiol Rev 57(3):543–594

Quiroz-Rocha E, Moreno R, Hernández-Ortíz A et al (2017) Glucose uptake in *Azotobacter vinelandii* occurs through a GluP transporter that is under the control of the CbrA/CbrB and Hfq-Crc systems. Sci Rep 7:858. https://doi.org/10.1038/s41598-017-00980-5

Rajput MS, Naresh Kumar G, Rajkumar S (2013) Repression of oxalic acid-mediated mineral phosphate solubilization in rhizospheric isolates of *Klebsiella pneumoniae* by succinate. Arch Microbiol 195:81–88. https://doi.org/10.1007/s00203-012-0850-x

Rajput MS, Iyer B, Pandya M et al (2015) Derepression of mineral phosphate solubilization phenotype by insertional inactivation of iclR in *Klebsiella pneumoniae*. PLoS ONE 10:e0138235. https://doi.org/10.1371/journal.pone.0138235

Remans K, Vercammen K, Bodilis J et al (2010) Genome-wide analysis and literature-based survey of lipoproteins in *Pseudomonas aeruginosa*. Microbiology 156:2597–2607. https://doi.org/10.1099/mic.0.040659-0

Rentz JA, Alvarez PJ, Schnoor JL (2004) Repression of *Pseudomonas putida* phenanthrene-degrading activity by plant root extracts and exudates. Environ Microbiol 6:574–583. https://doi.org/10.1111/j.1462-2920.2004.00589.x

Rojo F (2010) Carbon catabolite repression in *Pseudomonas* : optimizing metabolic versatility and interactions with the environment. FEMS Microbiol Rev 34:658–684. https://doi.org/10.1111/j.1574-6976.2010.00218.x

Ruiz-Manzano A, Yuste L, Rojo F (2005) Levels and activity of the *Pseudomonas putida* global regulatory protein Crc vary according to growth conditions. J Bacteriol 187:3678–3686. https://doi.org/10.1128/jb.187.11.3678-3686.2005

Sánchez-Hevia DL, Yuste L, Moreno R et al (2018) Influence of the Hfq and Crc global regulators on the control of iron homeostasis in *Pseudomonas putida*. Environ Microbiol 20:3484–3503. https://doi.org/10.1111/1462-2920.14263

Santiago-Frangos A, Woodson SA (2018) Hfq chaperone brings speed dating to bacterial sRNA. Wiley Interdiscip Rev RNA 9:e1475. https://doi.org/10.1002/wrna.1475

Sauer E, Weichenrieder O (2011) Structural basis for RNA 3'-end recognition by Hfq. Proc Natl Acad Sci USA 108:13065–13070. https://doi.org/10.1073/pnas.1103420108

Seshasayee AS, Bertone P, Fraser GM et al (2006) Transcriptional regulatory networks in bacteria: from input signals to output responses. Curr Opin Microbiol 9:511–519. https://doi.org/10.1016/j.mib.2006.08.007

Shimizu K (2014) Regulation systems of bacteria such as *Escherichia coli* in response to nutrient limitation and environmental stresses. Metabolites 4:1–35. https://doi.org/10.3390/metabo4010001

Silby MW, Winstanley C, Godfrey SA et al (2011) *Pseudomonas* genomes: diverse and adaptable. FEMS Microbiol Rev 35:652–680. https://doi.org/10.1111/j.1574-6976.2011.00269.x

Sonnleitner E, Blasi U (2014) Regulation of Hfq by the RNA CrcZ in *Pseudomonas aeruginosa* carbon catabolite repression. PLOS Genet 10:e1004440. https://doi.org/10.1371/journal.pgen.1004440

Sonnleitner E, Schuster M, Sorger-Domenigg T et al (2006) Hfq-dependent alterations of the transcriptome profile and effects on quorum sensing in *Pseudomonas aeruginosa*. Mol Microbiol 59:1542–1558. https://doi.org/10.1111/j.1365-2958.2006.05032.x

Sonnleitner E, Abdou L, Haas D (2009) Small RNA as global regulator of carbon catabolite repression in *Pseudomonas aeruginosa*. Proc Natl Acad Sci USA 106:21866–21871. https://doi.org/10.1073/pnas.0910308106

Sonnleitner E, Romeo A, Blasi U (2012a) Small regulatory RNAs in *Pseudomonas aeruginosa*. RNA Biol 9:364–371. https://doi.org/10.4161/rna.19231

Sonnleitner E, Valentini M, Wenner N et al (2012b) Novel targets of the CbrAB/Crc carbon catabolite control system revealed by transcript abundance in *Pseudomonas aeruginosa*. PLoS ONE 7:e44637. https://doi.org/10.1371/journal.pone.0044637

Sonnleitner E, Prindl K, Bläsi U (2017) The *Pseudomonas aeruginosa* CrcZ RNA interferes with Hfq-mediated riboregulation. PLoS One 12(7):e0180887. https://doi.org/10.1371/journal.pone.0180887

Sonnleitner E, Pusic P, Krennmayr B et al (2018a) Harnessing metabolic regulation to increase Hfq-dependent antibiotic susceptibility in *Pseudomonas aeruginosa*. Front Microbiol 9:2709. https://doi.org/10.3389/fmicb.2018.02709

Sonnleitner E, Wulf A, Campagne S et al (2018b) Interplay between the catabolite repression control protein Crc, Hfq and RNA in Hfq-dependent translational regulation in *Pseudomonas aeruginosa*. Nucleic Acids Res 46:1470–1485. https://doi.org/10.1093/nar/gkx1245

Storz G, Vogel J, Wassarman K (2011) Regulation by small RNAs in bacteria: expanding frontiers. Mol Cell 43:880–891. https://doi.org/10.1016/j.molcel.2011.08.022

Torres-Quesada O, Oruezabal RI, Peregrina A et al (2010) The *Sinorhizobium meliloti* RNA chaperone Hfq influences central carbon metabolism and the symbiotic interaction with alfalfa. BMC Microbiol 10:71. https://doi.org/10.1186/1471-2180-10-71

Tsipa A, Koutinas M, Vernardis SI et al (2017) The impact of succinate trace on pWW0 and ortho-cleavage pathway during transcription in *Pseudomonas putida* mt-2 toluene biodegradation. Biores Technol 234:397–405. https://doi.org/10.1016/j.biortech.2017.03.082

Udaondo Z, Ramos JL, Segura A et al (2018) Regulation of carbohydrate degradation pathways in *Pseudomonas* involves a versatile set of transcriptional regulators. Microb Biotechnol 11(3):442–454. https://doi.org/10.1111/1751-7915.13263

Updegrove TB, Zhang A, Storz G (2016) Hfq: the flexible RNA matchmaker. Curr Opin Microbiol 30:133–138. https://doi.org/10.1016/j.mib.2016.02.003

Valentini M, Garcia-Maurino SM, Perez-Martinez I et al (2014) Hierarchical management of carbon sources is regulated similarly by the

CbrA/B systems in *Pseudomonas aeruginosa* and *Pseudomonas putida*. Microbiology 160:2243–2252. https://doi.org/10.1099/mic.0.078873-0

Vincent HA, Henderson CA, Stone CM et al (2012) The low-resolution solution structure of *Vibrio cholerae* Hfq in complex with Qrr1 sRNA. Nucleic Acids Res 40:8698–8710. https://doi.org/10.1093/nar/gks582

Vogel J, Luisi BF (2011) Hfq and its constellation of RNA. Nat Rev Microbiol 9:578–589. https://doi.org/10.1038/nrmicro2615

von Bodman SB, Willey JM, Diggle SP (2008) Cell–cell communication in bacteria: united we stand. J Bacteriol 190:4377. https://doi.org/10.1128/JB.00486-08

Wang W, Shao Z (2013) Enzymes and genes involved in aerobic alkane degradation. Front Microbiol 4:116. https://doi.org/10.3389/fmicb.2013.00116

Wilf NM, Reid AJ, Ramsay JP et al (2013) RNA-seq reveals the RNA binding proteins, Hfq and RsmA, play various roles inc virulence, antibiotic production and genomic flux in *Serratia* sp. ATCC 39006. BMC Genom 14:1. https://doi.org/10.1186/1471-2164-14-822

Wirebrand L, Madhushani AWK, Irie Y et al (2018) Multiple Hfq-Crc target sites are required to impose catabolite repression on (methyl) phenol metabolism in *Pseudomonas putida* CF600. Environ Microbiol 20:186–199. https://doi.org/10.1111/1462-2920.13966

Wolff JA, MacGregor CH, Eisenberg RC et al (1991) Isolation and characterization of catabolite repression control mutants of *Pseudomonas aeruginosa* PAO. J Bacteriol 173:4700–4706. https://doi.org/10.1128/jb.173.15.4700-4706.1991

Yang N, Lan L (2016) *Pseudomonas aeruginosa* Lon and ClpXP proteases: roles in linking carbon catabolite repression system with quorum-sensing system. Curr Genet 62(1):1–6. https://doi.org/10.1007/s00294-015-0499-5

Yang N, Ding S, Chen F, Zhang X (2015) The Crc protein participates in down-regulation of the Lon gene to promote rhamnolipid production and rhl quorum sensing in *Pseudomonas aeruginosa*. Mol Microbiol 96:526–547. https://doi.org/10.1111/mmi.12954

Yeung AT, Bains M, Hancock RE (2011) The sensor kinase CbrA is a global regulator that modulates metabolism, virulence, and antibiotic resistance in *Pseudomonas aeruginosa*. J Bacteriol 193:918–931. https://doi.org/10.1128/jb.00911-10

Yuste L, Rojo F (2001) Role of the crc gene in catabolic repression of the *Pseudomonas putida* GPo1 alkane degradation pathway. J Bacteriol 183:6197–6206. https://doi.org/10.1128/jb.183.21.6197-6206.2001

Zhang XX, Rainey PB (2007) Genetic analysis of the histidine utilization (*hut*) genes in *Pseudomonas fluorescens* SBW25. Genetics 176:2165–2176. https://doi.org/10.1534/genetics.107.075713

Zhang XX, Rainey PB (2008) Dual involvement of CbrAB and NtrBC in the regulation of histidine utilization in *Pseudomonas fluorescens* SBW25. Genetics 178:185–195. https://doi.org/10.1534/genetics.107.081984

Zhang A, Wassarman KM, Ortega J et al (2002) The Sm-like Hfq protein increases OxyS RNA interaction with target mRNAs. Mol Cell 9:11–22. https://doi.org/10.1016/S1097-2765(01)00437-3

Zhang L, Gao Q, Chen W et al (2014) Regulation of *pqs* quorum sensing via catabolite repression control in *Pseudomonas aeruginosa*. Microbiology 159:1931–1936. https://doi.org/10.1099/mic.0.066266-0

Zimmermann T, Sorg T, Siehler SY et al (2009) Role of *Acinetobacter baylyi* Crc in catabolite repression of enzymes for aromatic compound catabolism. J Bacteriol 191:2834–2842. https://doi.org/10.1128/jb.00817-08