Carbon catabolite repression in bacteria: many ways to make the most out of nutrients

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Abstract | Most bacteria can selectively use substrates from a mixture of different carbon sources. The presence of preferred carbon sources prevents the expression, and often also the activity, of catabolic systems that enable the use of secondary substrates. This regulation, called carbon catabolite repression (CCR), can be achieved by different regulatory mechanisms, including transcription activation and repression and control of translation by an RNA-binding protein, in different bacteria. Moreover, CCR regulates the expression of virulence factors in many pathogenic bacteria. In this Review, we discuss the most recent findings on the different mechanisms that have evolved to allow bacteria to use carbon sources in a hierarchical manner.

Diauxie
The sequential use of carbon sources in a mixture of two different substrates. A short lag phase in the growth curve before the use of the less-preferred substrate is typical for diauxic growth.

Most bacteria can use various compounds as sources of carbon. These carbon sources can either be co-metabolized or the bacteria can preferentially use the carbon sources that are most easily accessible and allow fastest growth. One such example of selective carbon-source usage is the glucose–lactose diauxie in *Escherichia coli*, which was first observed by Jacques Monod[1] in 1942. Subsequent research with bacteria and higher organisms has revealed that selective carbon-source utilization is common and that glucose is the preferred carbon source in many of the model organisms that have been studied. Moreover, the presence of glucose often prevents the use of other, secondary, carbon sources. This preference for glucose over other carbon sources has been termed glucose repression or, more generally, carbon catabolite repression (CCR)[2]. Today, we define CCR as a regulatory phenomenon by which the expression of functions for the use of secondary carbon sources and the activities of the corresponding enzymes are reduced in the presence of a preferred carbon source.

CCR is one of the most important regulatory phenomena in many bacteria: as many as 5–10% of all bacterial genes are subject to CCR[3–6]. CCR is important for competition in natural environments, as selection of the preferred carbon source is a major determining factor in growth rate and therefore competitive success with other microorganisms. Moreover, CCR has a crucial role in the expression of virulence genes, which often enable bacteria to access new sources of nutrients. The ability to select the carbon source that allows fastest growth is the driving force for the evolution of CCR both in free-living and pathogenic bacteria.

CCR is observed in most free-living heterotrophic bacteria, including facultatively autotrophic bacteria that repress the genes for carbon dioxide fixation in the presence of organic carbon sources[7]. However, there are exceptions. Some pathogenic bacteria, such as *Chlamydia trachomatis* and *Mycoplasma pneumoniae*, which are highly adapted to nutrient-rich host environments, seem to lack CCR[8,9]. These organisms have small genomes and are adapted to only a few habitats and therefore lack most regulatory phenomena. Another peculiarity is the co-fermentation of glucose and other carbon sources that occurs in *Corynebacterium glutamicum*, although this co-fermentation is highly regulated[10,11]. Finally, for some bacteria, such as *Streptococcus thermophilus*, *Bifidobacterium longum* and *Pseudomonas aeruginosa*, glucose is only a secondary carbon source, and the genes for glucose utilization are repressed as long as the preferred carbon sources are available. This phenomenon, which is referred to as reverse CCR[12–14], will not be discussed in detail in this Review.

In many organisms, CCR of catabolic genes is achieved by the combined activities of global and operon-specific regulatory mechanisms. In this Review, we provide an overview of these mechanisms and discuss findings about CCR from different groups of

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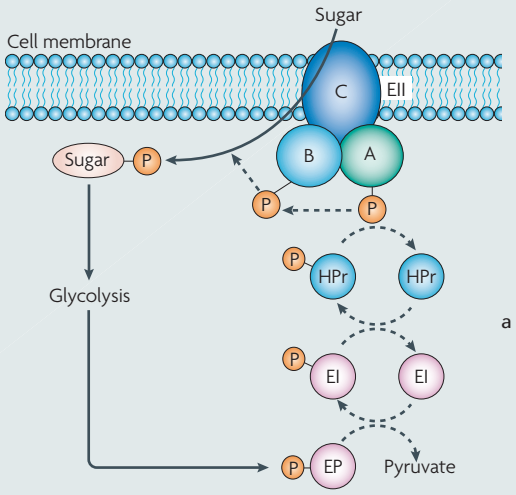
doi:10.1038/nrmicro1932

Box 1 | **The phosphoenolpyruvate–carbohydrate phosphotransferase system (PTS)**



The PTS is a multiprotein phosphorelay system that couples the transport of carbohydrates across the cytoplasmic membrane with their simultaneous phosphorylation[20] (see the figure). This type of active transport exists exclusively in bacteria. The PTS is composed of at least three distinct proteins that can be fused or encoded separately: enzyme I (EI), histidine protein (HPr) and enzyme II (EII). EI initiates the phosphorylation chain by autophosphorylating with phosphoenolpyruvate (PEP), and the phosphoryl group is subsequently transferred to the His15 residue in HPr. HPr then donates the phosphoryl group to a histidine residue in the A domains of the various substrate-specific transporters or EIIs. Finally, the phosphoryl group is transferred to a residue in the EIIB domain and from there to the carbohydrate during its uptake through the membrane domain (domain C).

All phosphoryl transfer reactions between PTS proteins are reversible. Therefore, the phosphorylation states of all PTS proteins that are present at a certain time is determined by two factors: PTS transport activity and the PEP to pyruvate ratio, which reflects flux through glycolysis. This dynamic modulation of the phosphorylation states of PTS proteins in response to nutritional conditions and the metabolic state of the cell provides the basis for PTS-mediated signalling and regulation.

Whereas the EIIA domain of the glucose transporter ($EIIA^{Glc}$) triggers carbon catabolite repression (CCR) in enterobacteria, HPr performs this function in Firmicutes. In addition, HPr(His-P) controls the activities of metabolic enzymes and transcriptional regulators by modulating their phosphorylation, and therefore contributes to CCR. EI and some EIIs have regulatory functions: non-phosphorylated EI mediates chemotaxis towards PTS substrates and some EIIs regulate the activities of their cognate transcriptional regulatory proteins by phosphorylation.

Interestingly, a comparative genome analysis suggests that the PTS primarily represents a signal-transduction system and that its transport function was acquired later in evolution[22].

bacteria[15–19]. In addition, we discuss the implications of CCR to the virulence of bacterial pathogens and modern approaches to study CCR in the context of systems biology.

Global regulation of CCR

CCR has been most intensively studied in the model organisms *E. coli* and *Bacillus subtilis*. In both species, CCR involves a global mechanism and several operon-specific regulatory mechanisms, such as inducer exclusion and induction prevention (discussed below). In *E. coli* and *B. subtilis*, the regulatory outcomes of the global mechanisms of CCR are similar: the genes that enable the use of secondary carbon sources are not expressed when glucose, or another preferred carbon source, is available. The molecular mechanisms by which this global regulation is achieved, however, are completely different. In *E. coli*, CCR is mediated by the prevention of transcriptional activation of catabolic genes in the presence of glucose. By contrast, in *B. subtilis*, CCR is mediated by negative regulation through a repressor protein in the presence of glucose. Although the mechanisms of CCR differ in these two organisms, the phosphoenolpyruvate–carbohydrate phosphotransferase system (PTS)[20–22] (BOX 1) is important in both organisms in the signal-transduction pathways that lead to CCR.

***CCR in* E. coli.** The major players in the global pathway of CCR in *E. coli* are the transcription activator CRP (cyclic AMP (cAMP) receptor protein; also called catabolite gene-activator protein (CAP)), the signal metabolite cAMP, adenylate cyclase and the IIA component of the glucose-specific PTS ($EIIA^{Glc}$; also called catabolite repression resistance (Crr) or $EIIA^{Crr}$) (FIG. 1).

Regulation of CCR is brought about by the modulation of the phosphorylation state of $EIIA^{Glc}$. In the presence of glucose or other PTS substrates, the phosphate from $EIIA^{Glc}$ is drained towards the sugars. The availability of the phosphate donor of the PTS, phosphoenolpyruvate (PEP), is also important. If the concentration ratio between PEP and pyruvate is high, $EIIA^{Glc}$ is predominantly phosphorylated. By contrast, if there are high amounts of pyruvate but low amounts of PEP in the cell, then $EIIA^{Glc}$ is predominantly dephosphorylated. $EIIA^{Glc}$ is preferentially dephosphorylated when *E. coli* cells grow rapidly with carbon sources that can be readily metabolized, such as glucose[23,24]. Phosphorylated $EIIA^{Glc}$ exerts its regulatory role by activating the membrane-bound enzyme adenylate cyclase. There is compelling genetic evidence to support this function of $EIIA^{Glc}$: cAMP levels are low in all mutants that are unable to form phosphorylated $EIIA^{Glc}$ (REFS 25,26).

The interaction of an artificially membrane-tethered adenylate cyclase with $EIIA^{Glc}$ has recently been studied. Both the phosphorylated and the unphosphorylated forms of $EIIA^{Glc}$ were found to interact with the carboxy (C)-terminal domain of adenylate cyclase without stimulating cAMP synthesis. Moreover, the phosphorylated, but not the unphosphorylated, form of $EIIA^{Glc}$ was able to activate adenylate cyclase activity in the presence of

Inducer exclusion
A mechanism of carbon catabolite repression by which the uptake or formation of the inducer of a catabolic operon is prevented in the presence of preferred carbon sources.

Induction prevention
A mechanism of carbon catabolite repression by which the activity of PTS-regulation-domain-containing transcription factors is inhibited in the presence of preferred carbon sources.

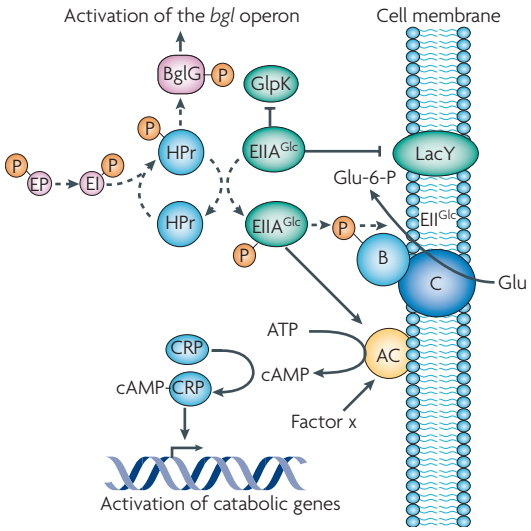


Figure 1 | **Carbon catabolite repression (CCR) in *Escherichia coli*.** The EIIA domain of the glucose transporter ($EIIA^{Glc}$) is the central processing unit in CCR in *E. coli*. When phosphorylated, $EIIA^{Glc}$ binds and activates adenylate cyclase (AC), which leads to cyclic AMP (cAMP) synthesis. An unknown 'factor x' is also required for the activation of AC. High cAMP concentrations trigger the formation of cAMP–CRP complexes, which bind and activate the promoters of catabolic genes. In its non-phosphorylated form, $EIIA^{Glc}$ cannot activate AC. In this case, $EIIA^{Glc}$ binds and inactivates metabolic enzymes and transporters of secondary carbon sources, such as GlpK, LacY and other proteins (not shown). This mechanism is called inducer exclusion because it prevents the intracellular formation of the inducer of the respective catabolic system. The phosphorylation state of $EIIA^{Glc}$ is determined by phosphoenolpyruvate–carbohydrate phosphotransferase system (PTS) transport activity (the presence of any PTS sugar results in dephosphorylation of $EIIA^{Glc}$) and the intracellular phosphoenolpyruvate (PEP) to pyruvate ratio, which decreases during high fluxes through glycolysis[24]. Histidine protein (HPr) also contributes to CCR by phosphorylating BglG, a transcriptional antiterminator protein that controls the expression of the β-glucoside utilization genes. Therefore, in the presence of preferred PTS sugars, the *bgl* operon is not expressed. The dashed arrows show phosphate transfer. Glu-6-P, glucose-6-phosphate.

E. coli cell extract[27]. Once cAMP has been synthesized by adenylate cyclase, cAMP binds CRP, its receptor protein, and the cAMP–CRP complex activates the promoters of many catabolic genes and operons. These promoters are usually weak and must be activated to allow the binding of RNA polymerase or the formation of the open transcription complex[28–30]. Interestingly, the cAMP–CRP complex not only mediates CCR of protein-coding genes, but also of small non-coding regulatory RNAs, such as the Spot42 and CyaR RNAs in *E. coli*[31,32]. This could be one explanation for how the cAMP–CRP complex has many indirect effects on gene expression.

Biochemistry and microbiology text-books usually present a simple correlation between the concentrations of glucose and cAMP (BOX 2): if a lot of glucose is present, there is only a little cAMP, and *vice versa*. However, an in-depth study of the CCR of the *E. coli lac* operon revealed that cAMP levels during growth with glucose or lactose are actually similarly low under both conditions[33]. These low amounts of cAMP are obviously sufficient to allow expression of the *lac* operon. The PEP to pyruvate ratio is the key factor that controls $EIIA^{Glc}$ phosphorylation, which explains why non-PTS carbohydrates, such as lactose, can also cause dephosphorylation of $EIIA^{Glc}$, resulting in low cAMP pools[23,24]. If cAMP concentrations are similarly low during growth with glucose or lactose, the obvious question is how does the formation of cAMP contribute to CCR at all? As discussed below, the operon-specific mechanism of inducer exclusion is the decisive factor for the glucose–lactose diauxie[33,34]. So is there any role for cAMP–CRP in CCR of the *E. coli lac* operon? The cAMP–CRP complex is required for expression of the *lac* operon, and it is also involved in CCR by activating expression of the EIIBC domain of the glucose-specific PTS and therefore the transport of glucose[35].

***CCR in* B. subtilis.** In the model firmicute *B. subtilis*, the pleiotropic transcription factor CcpA (catabolite control protein A), the HPr protein of the PTS, the bifunctional HPr kinase/phosphorylase (HPrK) and the glycolytic intermediates fructose-1,6-bisphosphate and glucose-6-phosphate are the key players in CCR[17,36,37] (FIG. 2).

Whereas the phosphorylation state of $EIIA^{Glc}$ is crucial for CCR in *E. coli*, HPr phosphorylation is central to CCR-related signal transduction in *B. subtilis*. In *B. subtilis*, HPr can be phosphorylated at a regulatory site, Ser46, and, in an EI-dependent manner, at His15 (BOX 1). HPr(Ser-P) serves as the effector for the dimeric CcpA protein and triggers its binding to operator sites, thereby causing transcriptional regulation[38,39]. The regulatory phosphorylation of HPr is catalysed by HPrK, a homohexameric enzyme that binds ATP by a classical P-loop motif[40]. HPrK kinase activity is triggered by the availability of fructose-1,6-bisphosphate as an indicator of high glycolytic activity[41–43]. By contrast, phosphorylase activity prevails under conditions of nutrient limitation, and this activity is stimulated by the accumulation of inorganic phosphate in the cell[43,44]. Thus, under conditions of good nutrient supply, HPrK acts as a kinase and HPr(Ser-P), the cofactor for CcpA, is formed.

Binding of HPr(Ser-P) to CcpA results in a slight rotational movement of the amino (N)-terminal and C-terminal subdomains of the CcpA core[45]. This movement brings the N-terminal DNA-binding domain of CcpA into a position that is competent for DNA binding. In addition to the contacts made by the phosphorylated Ser46 of HPr, His15 also contributes to the interaction with CcpA. This explains why phosphorylation of His15 inhibits CCR[46]. The interaction between CcpA and HPr(Ser-P) is enhanced by the glycolytic intermediates fructose-1,6-bisphosphate and glucose-6-phosphate[47,48]. Binding of these intermediates supports the interaction between an arginine residue of HPr(Ser-P) with two aspartate residues of the other subunit of the CcpA dimer[48].

P-loop motif
A phosphate-binding loop in many ATP- and GTP-binding proteins. The P-loop is composed of a glycine-rich sequence that is followed by a lysine and a serine or a threonine.

Box 2 | **Challenges to the model of catabolite repression**

Recent findings, from various experiments, have provided novel insight into carbon catabolite repression (CCR) of the *E. coli* lactose operon that has challenged the conventional information found in biology text-books. Below, we list some key questions for which the text-books are outdated.

What is the main contributor to CCR of the *lac* operon?
According to most text-books, it is the cyclic AMP (cAMP)–CRP complex that activates transcription in the absence of glucose. However, recent findings have revealed that inducer exclusion by inactivation of the lactose permease in the presence of glucose is the main contributor[33,126].

What is the main role of cAMP–CRP in CCR?
Text-books explain that cAMP–CRP predominantly affects the activation of the *lac* operon promoter. Although this is indeed the case, novel insight indicates that the main contribution of this complex to CCR and diauxie is the regulation of the glucose transporter gene *ptsG*[35].

How do the cAMP levels compare during growth with glucose or lactose?
Text-books state that cAMP levels are low during growth with glucose and are high during growth with lactose. However, experimental data indicate that cAMP concentrations are similar under both conditions[24,33].

How does glucose affect the *lac* operon in a mutant that lacks the Lac repressor?
This question has not been addressed previously. Recent findings now indicate, however, that there is no glucose repression in these mutants, as the inducer is not required in the absence of the repressor[33].

Another cofactor that could trigger the DNA-binding activity of CcpA is the Crh protein, a homologue of HPr. Crh does not contain a His15 residue, and has an exclusively regulatory function that depends on its HPrK-mediated phosphorylation of Ser46 (REF. 49). Crh(Ser-P) can replace HPr(Ser-P) in the complex with CcpA and can therefore trigger CCR[49–51]. However, the functional relevance of these observations has remained controversial, as the expression of Crh is much weaker than that of HPr and the affinity of CcpA for Crh(Ser-P) is reduced by ten-fold compared with the affinity for HPr(Ser-P)[52,53]. Finally, CcpA–Crh(Ser-P) complex formation is not stimulated by the presence of glycolytic intermediates[47], and it is therefore tempting to speculate that Crh is involved in signalling the nutrient status of the cell, although its cognate target remains unknown.

To cause CCR, the CcpA–cofactor complex must bind specific palindromic operator sequences in the promoter regions of catabolic operons that are called catabolite responsive elements (*cre*)[54,55]. Most *cre* sites are located in the transcription-initiation regions or overlap with the promoter consensus sequence[54]. However, in a few cases, such as the arabinose operon or the *sigL* gene, *cre* sites can also be located further downstream, and binding of CcpA to such sites might cause a roadblock for the RNA polymerase[56,57]. Intriguingly, there are cases in which CcpA binds upstream of the promoter. However, in these cases, CcpA activates rather than represses transcription. This was observed for the *ackA* and *pta* genes, the products of which are required for the excretion of acetate when cells are grown in the presence of excess carbon[58,59].

In addition to direct regulation by the binding of CcpA to a *cre* site in the target gene, there are many examples of indirect regulation by CcpA[4]. Indirect regulation can be caused by strongly reduced glucose transport by the PTS in *ccpA* mutants or by insufficient accumulation of inducers. For example, acetate, the inducer of the *alsSD* operon that is involved in acetoin synthesis, is not produced in a *ccpA* mutant, and therefore the *alsSD* operon cannot be expressed in such mutants[60,61].

Lactose permease
The best-studied transport protein. Lactose permease concomitantly transports lactose and protons into the cell (symport). Uptake of these protons provides the energy for lactose transport and accumulation.

Catabolite control of operon-specific induction

In addition to global mechanisms of CCR, there are also operon-specific mechanisms. These mechanisms address either the formation or uptake of the operon-specific inducer (inducer exclusion) or the activity of operon-specific transcription factors (induction prevention). Both mechanisms have been described in *E. coli* and *B. subtilis*. The best-studied example of inducer exclusion is the *E. coli* lactose operon, and the best-studied example of induction prevention is the *B. subtilis bglPH* operon. CCR of glycerol kinase activity in *E. coli* and *B. subtilis*, another well-studied example, has recently been reviewed[19].

Catabolite control of sugar transporters. The *E. coli lac* operon is only expressed if allolactose (a lactose isomer formed by β-galactosidase) binds and inactivates the *lac* repressor. Formation of allolactose requires the uptake of at least some lactose. However, lactose cannot be transported into the cell in the presence of glucose, because the lactose permease, LacY, is inactive in the presence of glucose[62]. $EIIA^{Glc}$ has a key role in the control of the activity of LacY: in the absence of glucose, $EIIA^{Glc}$ is phosphorylated and does not interact with LacY, whereas in the presence of glucose, non-phosphorylated $EIIA^{Glc}$ can bind and inactivate LacY[34,63] (FIG. 1). Interestingly, this interaction only occurs if lactose is present[64].

The formation of a complex of periplasmic lactose, the membrane-spanning lactose permease and the cytoplasmic $EIIA^{Glc}$ is possible due to structural rearrangements that occur in LacY upon lactose binding. These rearrangements extend to the cytoplasmic side of LacY and allow the interaction with $EIIA^{Glc}$ (REF. 64). This is important, because the same mechanism also applies to the transport of other secondary carbon sources, such as maltose, melibiose, raffinose and galactose[65,66]. Control of the inhibitory interaction between $EIIA^{Glc}$ and specific permeases by the respective substrate of the transporter allows the available $EIIA^{Glc}$ to be directed to those permeases that really deserve to be inactivated at a particular time[67]. Inducer exclusion is the main reason for the glucose–lactose diauxie in *E. coli*[33].

Inducer exclusion has also been described for Gram-positive bacteria, and HPr is the major player in these organisms. In *Lactobacillus brevis*, HPr(Ser-P) that is formed in the presence of glucose binds and inactivates galactose permease[68]. By contrast, the activity of the *S. thermophilus* lactose permease is controlled by HPr(His-P)-dependent phosphorylation. This non-PTS permease contains a domain that is similar to the EIIA domains of the PTS. In the absence of glucose, HPr(His-P) can phosphorylate this PTS-like domain, thereby activating the permease for lactose transport[69]. If glucose is present, HPr becomes phosphorylated on Ser46 and can no longer activate the lactose permease[70].

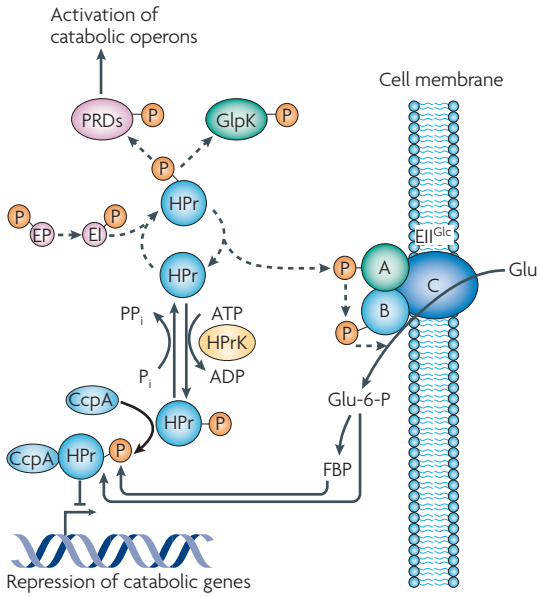


Figure 2 | **Carbon catabolite repression (CCR) in *Bacillus subtilis* and other Firmicutes.** In Firmicutes, histidine protein (HPr) can be phosphorylated at Ser46 by the HPr kinase/phosphorylase (HPrK). This phosphorylation occurs when the intracellular concentrations of fructose-1,6-bisphosphate (FBP) and ATP are high, which reflects the presence of preferred carbon sources. HPr(Ser-P) binds to the CcpA protein, and this interaction is enhanced by glycolytic intermediates, such as FBP and glucose-6-phosphate (Glu-6-P). The complex of CcpA and HPr(Ser-P) binds to *cre* sites on the DNA, and thereby represses the transcription of catabolic genes. HPrK is also responsible for dephosphorylation of HPr(Ser-P) under conditions of high inorganic phosphate (P_i) and low ATP, and when FBP concentrations reflect poor nutritional supply. In addition, HPr(His-P) contributes to CCR: in the absence of glucose, HPr(His-P) phosphorylates the glycerol kinase GlpK and transcriptional regulators that contain phosphoenolpyruvate–carbohydrate phosphotransferase system-regulatory domains (PRDs), which is a prerequisite for their activity. Thus, in the presence of glucose, activation of the PRD regulators by their inducers is prevented, a mechanism that has been called induction prevention. The dashed arrows show phosphate transfer.

PTS-regulatory domain
(PRD). A protein domain of ~100 amino acids that is found in many transcription regulators and that is always present in duplicate in PRD-containing regulators. These domains can be phosphorylated by PTS components (either the sugar-specific EIIB or HPr) on conserved histidine residues and contribute to substrate induction and catabolite repression. The phosphorylation state of the PRDs determines the activity of the regulator.

Antitermination
A regulatory mechanism by which the formation of a transcription terminator is prevented under inducing conditions. Many catabolic operons are controlled by protein-dependent antitermination systems that result in transcription elongation into the structural genes only in the presence of the substrate.

Cellulolytic clostridia
Obligately anaerobic, spore-forming bacteria that can degrade cellulose. Cellulolytic clostridia form an extracellular protein complex, called the cellulosome, that makes the cellulose fibres accessible to the cellulases that hydrolyse the cellulose.

Catabolite control of transcription factors. In both *E. coli* and *B. subtilis*, several operons for the catabolism of PTS substrates are controlled by transcription regulators that contain duplicated PTS-regulatory domains (PRDs). These regulators can act as transcription activators or as RNA-binding antitermination proteins. Their activity is modulated by the availability of their specific substrate and by the superimposed mechanism of CCR. The PRD-containing regulators perceive the information on substrate and glucose availability by PTS-dependent phosphorylation of their PRDs[71]. This type of regulation allows the hierarchical use of PTS sugars. Each of these regulatory systems consists of a PRD-containing transcription regulator and the sugar-specific cognate EII of the PTS.

PRD-mediated regulation has been studied most intensively for the *B. subtilis* LicT antiterminator, which controls expression of the *bglPH* operon for the use of aryl-β-glucosides (FIG. 3). In the absence of β-glucosides, LicT is phosphorylated by the β-glucoside-specific EII on its first PRD (PRD1). This phosphorylation results in the inactivation of LicT. In the presence of β-glucosides, the incoming sugars become phosphorylated, and LicT is dephosphorylated and therefore activated[72]. However, the activity of LicT is not only controlled by the specific substrate, but also by the availability of glucose or other PTS sugars. In the presence of glucose, LicT is inactive[73]. To become active, LicT not only needs to be dephosphorylated on its PRD1, but it must also be phosphorylated on its PRD2. The phosphorylation of PRD2 is catalysed by HPr(His-P), which is only available in the absence of glucose[60,74,75]. Thus, the phosphorylation state of HPr links the availability of PTS substrates to the activity of LicT (FIG. 3).

Glucose-regulated phosphorylation of PRDs has also been observed for other PRD-containing regulators, including the *E. coli* BglG antiterminator (FIG. 1) and the *B. subtilis* LevR transcription activator. Both proteins require HPr-dependent phosphorylation for full activity[76,77]. Interestingly, the *B. subtilis* GlcT antiterminator that controls glucose transport does not require HPr-dependent phosphorylation for activity, and is therefore active in the presence of glucose[78].

CCR in other bacteria

Whereas the mechanisms of CCR have been studied in detail in the model organisms *E. coli* and *B. subtilis*, less is known about CCR in other bacteria. Recent research indicates that each group of bacteria has evolved its own mechanism of CCR, as outlined below for the Firmicutes, Actinobacteria and pseudomonads.

CCR in other Firmicutes. With the exception of the mycoplasmas, all Firmicutes use the same elements of CCR as *B. subtilis*[17] — HPr, HPrK and CcpA. A transcriptomic analysis of the CcpA regulon of the lactic acid bacterium *Lactococcus lactis* revealed that CcpA represses not only genes of carbon metabolism, but also its own expression[79]. Importantly, CcpA in lactic acid bacteria controls the general routes of metabolism, such as glycolysis and lactic acid formation[12,79–81].

In cellulolytic clostridia, expression of the key components of the cellulosome[82] is subject to CCR[83]. For the *Clostridium cellulolyticum cip-cel* operon, this repression is caused by CcpA binding to *cre* sites in the promoter region. Interestingly, *C. cellulolyticum* does not contain a functional HPr or an EII. Instead, a protein that resembles *B. subtilis* Crh acts as the cofactor for CcpA[84].

CCR in actinobacteria. In actinobacteria, CCR has been studied in members of the genus *Streptomyces*, in *B. longum* and in *C. glutamicum*, but so far no common mechanisms of CCR have been discovered in this group of bacteria.

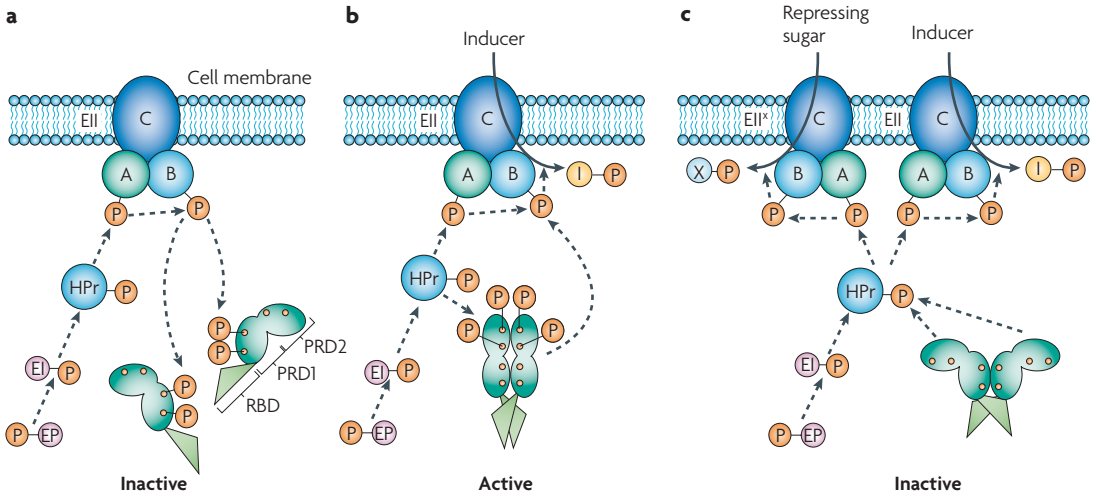


Figure 3 | **Regulation of LicT antitermination activity by HPr- and EII^{Bgl}-catalysed phosphorylations. a** | In the absence of phosphoenolpyruvate–carbohydrate phosphotransferase system (PTS) sugars, EII^{Bgl} phosphorylates LicT at the histidines in PTS regulatory domain 1 (PRD1). In addition, histidine protein (HPr) may phosphorylate LicT at the histidines in PRD2 (not shown). This multiple phosphorylated form of LicT cannot dimerize, and therefore is not active in antitermination. **b** | In the presence of inducing sugars (β-glucosides), EII^{Bgl} de-phosphorylates PRD1 of LicT. This promotes the formation of LicT dimers, which are proficient in antitermination of the *bglPH* transcripts. However, activation of LicT also requires HPr-catalysed phosphorylation of its PRD2. The phosphorylated histidines are buried at the interface. **c** | In the presence of additional PTS sugars, such as glucose, the concentration of HPr(His-P) drops and the phosphoryl groups at the PRD2 of LicT are therefore transferred back to HPr. In the non-phosphorylated state, the PRD2 swings out and only PRD1 is dimeric. Dimerization of the RNA-binding domain (RBD) could therefore be prevented and LicT would be unable to bind to its mRNA target[128,129]. This model might also be accurate for other PRD-containing regulators that are controlled by dual HPr- and EII-catalysed phosphorylations. The dashed arrows show phosphate transfer. EII^{x}, EII for the transport of a repressing sugar.

In *Streptomyces coelicolor*, CCR of genes that are involved in the use of secondary carbon sources seems to be independent of the PTS[85]. Instead, glucose kinase is the key player of CCR in *S. coelicolor* and related species. In *glk* mutants that lack this enzyme, neither glucose nor other readily usable carbon sources exert CCR. As glucose kinase is not involved in the metabolism of repressing sugars that are different from glucose, it was concluded that the biochemical state of glucose kinase, rather than the flux through glucose kinase, is important for CCR[86]. This conclusion is supported by the observation that a heterologous glucose kinase restores glucose utilization, but not CCR, in *S. coelicolor*[87]. Thus, glucose kinase has a direct regulatory role in *S. coelicolor* and belongs to the group of trigger enzymes that exert functions in gene regulation in addition to their catalytic activities[88]. It has been suggested that metabolite-activated glucose kinase directly interacts with operon-specific regulators, resulting in CCR of the respective catabolic operons[85,89,90].

The regulation of carbon metabolism in *C. glutamicum* has been extensively studied because of the importance of this bacterium in the biotechnological production of amino acids. *C. glutamicum* prefers to use multiple carbon sources simultaneously. Diauxic growth was observed for media that contain glutamate or ethanol in addition to glucose. In the presence of glucose, the repressor protein RamB is activated by an unknown mechanism and binds its target sites in the promoter regions of genes that are involved in acetate and ethanol catabolism[91,92]. Moreover, *ramB* expression is controlled by a feedback loop that includes RamB and a second regulatory protein, RamA[93]. RamA, in turn, is activated in the presence of acetate. This complex regulation ensures that RamB is available as a pathway-specific regulator of CCR if acetate, the substrate of the pathway, is present.

***CCR in* Pseudomonas putida *and* Acinetobacter baylyi.** Other than *E. coli* and the closely related enteric bacteria, CCR has been studied in detail only in two gammaproteobacteria, *Pseudomonas putida* and *Acinetobacter baylyi*, both of which use various different aromatic and aliphatic hydrocarbons. However, the general settings of carbon metabolism and the mechanisms of CCR in these bacteria are completely different from those found in *E. coli*.

In *P. putida* and *A. baylyi*, the use of hydrocarbon is repressed by succinate and, to a lesser extent, by glucose. The strong repression by succinate seems to be a general feature of CCR in these organisms[14,94]. Several studies indicate that CCR of the individual catabolic pathways is achieved by controlling expression of the operon-specific regulators. These regulators activate the expression of their target operons in the presence of the cognate inducer. However, under conditions of CCR, the translation of these regulators is inhibited by the binding of a globally acting RNA-binding protein, Crc, to the 5′ regions of the mRNAs of the regulator

Trigger enzyme
An enzyme that is involved in the control of gene expression in response to gene-activity states. These enzymes can exert their regulatory functions by acting as direct transcription factors (binding to DNA or RNA) or by controlling the activity of other transcription factors (by covalent modification or regulatory protein–protein interactions).

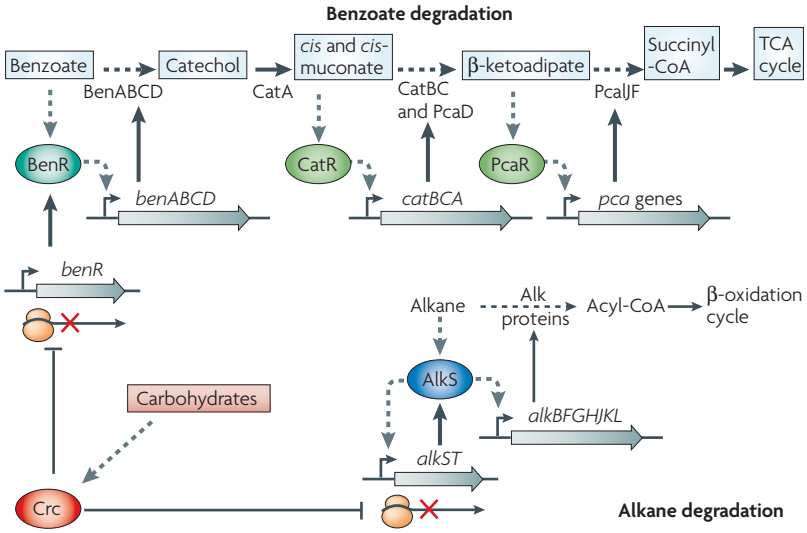


Figure 4 | **Carbon catabolite repression (CCR) by translational repression in *Pseudomonas putida*.** *P. putida* can degrade aromatic compounds, such as benzoate, and hydrocarbons, such as alkanes. In the presence of preferred carbon sources, such as succinate, the global regulatory protein Crc binds to the 5′ ends of the mRNAs that encode the BenR and AlkS transcriptional regulator proteins and inhibits their translation. These activator proteins are required for the substrate-dependent expression of the benzoate and alkane degradation genes, respectively. In the benzoate-degradation pathway, repression of the *ben* genes also causes repression of the *cat* and *pca* operons, which encode enzymes that catalyse reactions downstream of benzoate in the pathway. Repression of the *ben* genes prevents the formation of inducers for the regulatory proteins CatR and PcaR, which activate the expression of the *cat* and *pca* operons. Crc also has a role in the CCR of other catabolic systems, which suggests that it is a global regulator. It remains to be shown whether targeting translation of the corresponding regulatory proteins to repress catabolic pathways is a general strategy of Crc to exert CCR. The dashed arrows show phosphate transfer. TCA, tricarboxylic acid.

transcripts[95,96] (FIG. 4). Therefore, in these bacteria, CCR seems to be governed by an RNA-binding protein at the level of post-transcriptional control rather than by a DNA-binding transcription regulator.

The impact of CCR on bacterial virulence

In many pathogenic bacteria, elements of CCR are crucial for the expression of virulence genes and therefore for pathogenicity. It is important to keep in mind that the primary aim of pathogenic bacteria is to gain access to nutrients rather than to cause damage to the host. The proteins that are encoded by virulence genes are often involved in the use of alternative nutrients, and therefore it would make sense if the expression of virulence genes is linked to the nutrient supply of the bacteria.

Virulence in Firmicutes. In pathogenic bacteria of the genus *Streptococcus*, CcpA and CCR are important for the expression of virulence genes. In *S. pneumoniae*, CcpA is required for colonization of the nasopharynx and for survival and multiplication in the lung[97]. *S. pyogenes ccpA* mutants are considerably less virulent than wild-type strains[98]. Transcriptome studies have indicated that CcpA controls the expression of several virulence factors in response to the nutrient supply. Among these, the *sagA* operon, which is required for streptolysin S production, is strongly repressed by glucose in a CcpA-dependent manner[99]. By contrast, expression of the *speB* gene, which encodes a cysteine protease, and the *mac* gene, which encodes an immunoglobulin-degrading enzyme, is activated by CcpA[98]. Another level of virulence control by CcpA occurs in the regulation of the *S. pyogenes mga* gene. Mga is the master regulator of virulence genes in *S. pyogenes*, and its expression is activated by CcpA[100] (FIG. 5). Moreover, Mga contains two domains that resemble PRDs (discussed above) and might link the nutrient state of the cell to its activity[101]. In *S. mutans*, the causative agent of caries, virulence-related functions, such as expression of the fructanase gene, acid formation and acid tolerance, are subject to CcpA-dependent CCR[81,102]. Therefore, the potential of *S. mutans* to cause caries is governed in large part by CcpA.

In *Listeria monocytogenes*, expression of genes that are required for entering the host cell, release from the phagosome into the cytosol and intracellular and intercellular motility are controlled by the transcription activator PrfA[103]. The activity of PrfA is strongly inhibited if bacteria grow in the presence of glucose or other PTS substrates[104,105]. The mechanism that governs PrfA control by sugar availability is unknown, although the classical components, such as CcpA, HPrK and HPr(Ser-P), are not involved. Instead, PTS-dependent transport activity seems to be crucial for signalling to PrfA[105].

In *Clostridium perfringens*, the causative agent of severe histolytic diseases, glucose represses several virulence-associated processes, such as gliding motility and toxin production. The *pilT* and *pilD* genes, which encode pilus components for gliding, as well as the *cpe* gene, which encodes the major enterotoxin, are subject to CcpA-mediated CCR[106–108].

Virulence in proteobacteria. A relationship between CCR and virulence also exists in pathogenic proteobacteria. In the uropathogenic *E. coli* strain 536WT, the formation of S fimbriae is essential for adhesion to sialic acid-containing eukaryotic receptor molecules. Expression of the corresponding gene, *sfaA*, is subject to CCR and depends on activation by the cAMP–CRP complex[109]. Similarly, adhesion of the Vero cytotoxin-producing *E. coli* and haemolysin production of an avian pathogenic *E. coli* are repressed by glucose[110,111]. In serovars of *Salmonella enterica*, a key regulatory system of virulence, the BarA–SirA two-component system, is subject to cAMP–CRP-mediated CCR. The SirA response regulator, in turn, controls expression of a type III secretion system that is encoded on Salmonella pathogenicity island 1 (SPI1)[112], as well as expression of the regulatory RNAs CsrB and CsrC. CCR of SirA expression therefore provides a link between carbon supply and the expression of virulence genes that are encoded on SPI1 (REF. 113). This explains the attenuation of virulence of *S. enterica* that is caused by *crp* and *cya* mutations[114]. An essential role for cAMP and CRP in virulence was also observed

Pathogenicity island
A DNA region with clusters of genes that are involved in virulence functions. Pathogenicity islands are mobile genetic elements that are typically flanked by repeat structures and mobility genes that are required for horizontal gene transfer.

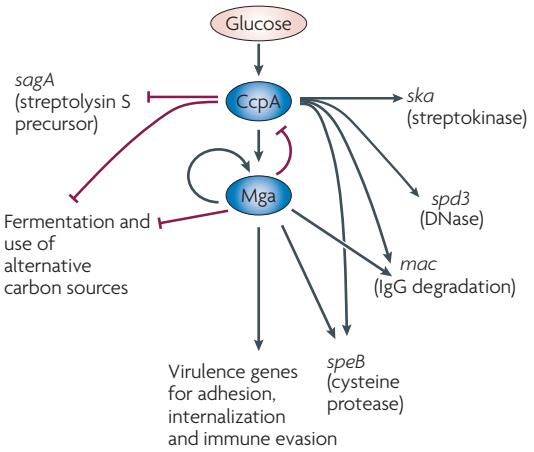


Figure 5 | **Interplay between CcpA-mediated carbon catabolite repression and virulence gene expression in *Streptococcus pyogenes*.** In addition to genes that are involved in carbohydrate utilization, CcpA directly controls the expression of several genes with important functions in the virulence of *S. pyogenes*. Moreover, CcpA activates the expression of *mga*, which encodes the master regulator of virulence. Mga, in turn, controls the expression of approximately 10% of all *S. pyogenes* genes. These genes are predominantly involved in sugar uptake and metabolism, and in specific virulence functions, such as adhesion and internalization in host cells and immune-system evasion. In addition, Mga activates its own expression and represses *ccpA* expression. The CcpA–Mga regulon allows *S. pyogenes* cells to activate virulence functions when new glucose-rich tissues of the host are colonized.

for *Vibrio cholerae* and *Yersinia enterocolitica*[115,116]. In *Yersinia pestis*, expression of the plasminogen activator gene requires activation by the cAMP–CRP complex. The plasminogen activator is essential both for the dissemination of *Y. pestis* from the site of the original infection and for the bacterium to spread from the lung to cause a systemic infection[117].

In the plant pathogen *Erwinia chrysanthemi*, the degradation of pectin, a major constituent of the plant cell wall, is a prerequisite for invasion into plant tissues and therefore for phytopathogenicity. As observed for major virulence determinants of animal and human pathogens, expression of the pectinases of *E. chrysanthemi* requires the cAMP–CRP complex and is repressed by glucose[118].

CCR and its implication in the treatment of infectious diseases. CCR is not only involved in the expression of virulence determinants in pathogenic bacteria, but also plays a part in the development of resistance to antibiotics. Moreover, components of CCR could represent novel antibacterial drug targets, and non-pathogenic mutants in which CCR has been modified could be used to develop novel live vaccines.

In *Staphylococcus aureus*, CcpA contributes to meticillin resistance and provides the highly meticillin-resistant strain *S. aureus* COLn with additional resistance to oxacillin[119,120]. Similarly, in *Streptococcus gordonii*, one of the causative agents of infective heart diseases, CcpA is important for tolerance to penicillin[121]. However, in both cases, the mechanisms that underlie the involvement of CcpA in resistance and tolerance remain unknown.

In many Firmicutes, mutants that are devoid of the HPr kinase grow significantly slower than wild-type cells. It was therefore suggested that HPr kinase, which generates the cofactor for CcpA, might be a suitable drug target. Indeed, a compound that inhibits the kinase activity of HPr has been identified. This compound inhibits the growth of *B. subtilis*, but not of *E. coli*. This is in agreement with the fact that *E. coli* does not contain an HPr kinase[122].

As mentioned above, CRP and cAMP are essential for the expression of virulence genes in enteric bacteria, and therefore the corresponding *crp* and *cya* mutant strains of *S. enterica* and *Y. enterocolitica* can be used as live vaccines in mice and pigs[115,123,124]. These mutants are promising candidates for the development of novel vaccines for livestock and, perhaps, also for humans.

CCR in the age of systems biology

As with any regulatory phenomenon, CCR depends on gene expression, protein activities and metabolite availabilities. To obtain a complete understanding of CCR, mathematical modelling of these different levels is required. The generation and validation of such models is the subject of the new field of systems biology. These models could enable us to predict whether CCR occurs under conditions that are difficult to assess experimentally.

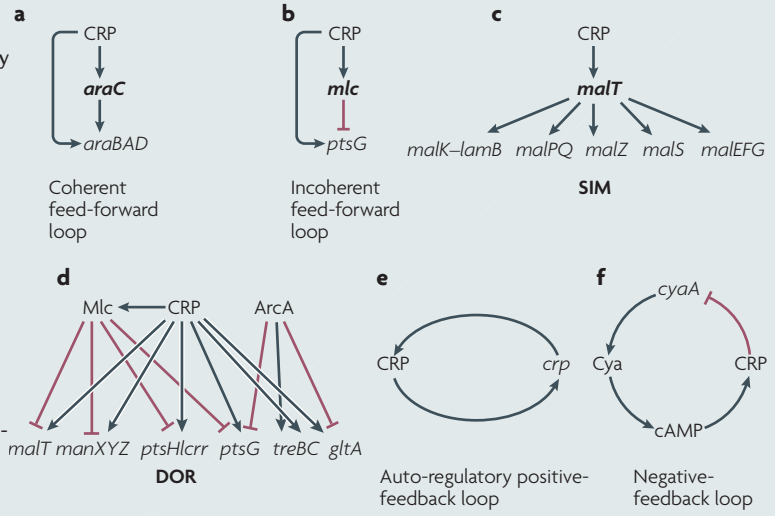
So far, modelling approaches have been applied only to CCR in *E. coli*[125,126]. To simulate and model complex regulatory networks, it is important to understand the basic building blocks (also called network motifs)[127] (BOX 3) of the system. A model for CCR of the *E. coli* operons for lactose and glycerol utilization not only includes the well-established players of CCR (FIG. 1), but also 'minor factors', such as the control of the amounts of general PTS proteins. Although the amount of these proteins is modulated only twofold, this regulation is important for the outcome of the model[126]. An important outcome predicted by the mathematical model was that CCR of glycerol utilization is mainly caused by transcription control through cAMP–CRP, rather than by glycerol-kinase-mediated inducer exclusion[126]. By contrast, as discussed above, inducer exclusion is the main mechanism in CCR of lactose utilization[33,126] (BOX 2, FIG. 1).

Although systems biology is still in its infancy, it has already provided important new insights into the mechanisms of CCR in *E. coli*. Further refinement and development of models for other organisms will stimulate the design of new experiments that would not have been planned without such a mathematical analysis.

Conclusions

CCR is one of the main regulatory phenomena in bacteria. CCR helps bacteria to make the most efficient use of the available carbon sources. This is true both for free-living organisms that are frequently faced with nutrient limitations and for pathogenic bacteria

Box 3 | **Motifs in carbon catabolite repression (CCR) regulatory networks**



Regulatory networks exhibit patterns of interconnections that occur statistically more frequently than they would in a randomized network, and are therefore named network motifs. The transcriptional regulation network of *Escherichia coli* consists of three motifs: feed-forward loops, single-input modules (SIMs) and dense overlapping regulons[127].

In feed-forward loops, a transcription factor regulates another transcription factor, and both transcription factors jointly regulate a target gene. Most feed-forward loops are coherent — similar to CCR of arabinose utilization, the action of both regulators has the same sign (see the figure for a comparison of a coherent feed-forward loop (**a**) and an incoherent feed-forward loop (**b**)). Expression of the *ara* operon occurs only when the inducing signal persists for a long enough time period to allow for the accumulation of a sufficient amount of AraC activator protein. Therefore, feed-forward loops do not respond to transient signals, a property that is called robustness. The SIM represents a motif in which a single transcription factor regulates a group of genes (see the figure, part **c**). This strategy allows the coordinated regulation of several operons. There are also dense overlapping regulons (DORs), in which a group of operons is regulated by several factors. Many of the genes that are regulated by CRP are also controlled by other global regulators (see the figure, part **d**). Similar to a computer, DORs integrate various signals to generate a complex output.

Interestingly, there are almost no feedback loops in the *E. coli* transcriptional network, except for simple auto-regulatory loops (see the figure, part **e**). However, such positive-feedback loops would lead to indefinite explosion, and therefore they must be controlled by negative-feedback loops. For example, CRP negatively regulates the expression of adenylate cyclase, which synthesizes cyclic AMP (cAMP), the cofactor that is required for CRP activity (see the figure, part **f**). This mechanism prevents the unlimited expression of CRP. The many regulatory feedback loops in CCR are exerted at the post-transcriptional level and involve protein–protein interactions, protein phosphorylation and the synthesis of effector metabolites.

that need to access the potential nutrients in their hosts. Because of this general importance, it is not surprising that CCR is found in nearly all bacteria. However, each group of bacteria has evolved its own way of achieving CCR.

Although the basic principles of CCR are well understood, at least in model organisms, many questions remain unanswered. Even for *E. coli*, we have not yet identified the 'factor x' that is required for the activation of adenylate cyclase by $EIIA^{Glc}$ (FIG. 1). For the other model organism, *B. subtilis*, elucidation of the function of the second protein that is phosphorylated by the HPrK, Crh, is high on the agenda.

For all non-model organisms, we are only beginning to understand CCR. For example, the signals that are perceived by Crc in *P. putida* or the regulatory pathway that involves the glucose kinase in *S. coelicolor* are urgent questions for further research. For a better understanding of the link between CCR and virulence, control of the PrfA transcription activator by the PTS in *L. monocytogenes* needs to be analysed, and this might even result in the discovery of a novel principal mechanism of CCR. Before we can use systems biology to study CCR in other bacteria, we must first address these issues.

CCR is the best-understood global regulatory phenomenon. Further analysis of CCR may help microbiologists to get deeper insights into a complex regulatory network. Moreover, the implications of CCR in virulence, which we are just beginning to understand, will be important for our views on the relationship between human, animal and plant hosts and pathogenic microorganisms.

1. Monod, J. *Recherches sur la Croissance des Cultures Bactériennes*. Thesis, Hermann et Cie, Paris (1942).
2. Magasanik, B. Catabolite repression. *Cold Spring Harb. Symp. Quant. Biol.* **26**, 249–256 (1961).
3. Liu, M. *et al.* Global transcriptional programs reveal a carbon source foraging strategy by *Escherichia coli*. *J. Biol. Chem.* **280**, 15921–15927 (2005).
4. Blencke, H.-M. *et al.* Transcriptional profiling of gene expression in response to glucose in *Bacillus subtilis*: regulation of the central metabolic pathways. *Metab. Eng.* **5**, 133–149 (2003).
5. Moreno, M. S., Schneider, B. L., Maile, R. R., Weyler, W. & Saier, M. H. Jr. Catabolite repression mediated by the CcpA protein in *Bacillus subtilis*: novel modes of regulation revealed by whole-genome analyses. *Mol. Microbiol.* **39**, 1366–1381 (2001).
6. Yoshida, K.-I. *et al.* Combined transcriptome and proteome analysis as a powerful approach to study genes under glucose repression in *Bacillus subtilis*. *Nucleic Acids Res.* **29**, 6683–6692 (2001).
7. Bowien, B. & Kusian, B. Genetics and control of CO_2 assimilation in the chemoautotroph *Ralstonia eutropha*. *Arch. Microbiol.* **178**, 85–93 (2002).
8. Nicholson, T. L., Chiu, K. & Stephens, R. S. *Chlamydia trachomatis* lacks an adaptive response to changes in carbon source availability. *Infect. Immun.* **72**, 4286–4289 (2004).

9. Halbedel, S. *et al.* Transcription in *Mycoplasma pneumoniae*: analysis of the promoters of the *ackA* and *Idh* genes. *J. Mol. Biol.* **371**, 596–607 (2007).
10. Frunzke, J., Engels, V., Hasenbein, S., Gätgens, C. & Bott, M. Co-ordinated regulation of gluconate catabolism and glucose uptake in *Corynebacterium glutamicum* by two functionally equivalent transcriptional regulators, GntR1 and GntR2. *Mol. Microbiol.* **67**, 305–322 (2008).
11. Wendisch, V. F., de Graaf, A. A., Sahm, H. & Eikmanns, B. J. Quantitative determination of metabolic fluxes during coutilization of two carbon sources: comparative analyses with *Corynebacterium glutamicum* during growth on acetate and/or glucose. *J. Bacteriol.* **182**, 3088–3096 (2000).
12. Van den Bogaard, P. T. C., Kleerebezem, M., Kuipers, O. P. & de Vos, W. M. Control of lactose transport, β-galactosidase activity, and glycolysis by CcpA in *Streptococcus thermophilus*: evidence for carbon catabolite repression by a non-phosphoenolpyruvate-dependent phosphotransferase system sugar. *J. Bacteriol.* **182**, 5982–5989 (2000).
13. Parche, S. *et al.* Lactose-over-glucose preference in *Bifidobacterium longum* NCC2705: *glcP*, encoding a glucose transporter, is subject to lactose repression. *J. Bacteriol.* **188**, 1260–1265 (2006).
14. Collier, D. N., Hager, P. W. & Phibbs, P. V. Jr. Catabolite repression control in the *Pseudomonads*. *Res. Microbiol.* **147**, 551–561 (1996).
15. Stülke, J. & Hillen, W. Carbon catabolite repression in bacteria. *Curr. Opin. Microbiol.* **2**, 195–201 (1999).
16. Brückner, R. & Titgemeyer, F. Carbon catabolite repression in bacteria: choice of the carbon source and autoregulatory limitation of sugar utilization. *FEMS Microbiol. Lett.* **209**, 141–148 (2002).
17. Titgemeyer, F. & Hillen, W. Global control of sugar metabolism: a Gram-positive solution. *Antonie van Leeuwenhoek* **82**, 59–71 (2002).
18. Kotrba, P., Inui, M. & Yukawa, H. Bacterial phosphotransferase system (PTS) in carbohydrate uptake and control of carbon metabolism. *J. Biosci. Bioeng.* **92**, 502–517 (2001).
19. Deutscher, J. The mechanisms of carbon catabolite repression in bacteria. *Curr. Opin. Microbiol.* **11**, 87–93 (2008).
20. Postma, P. W., Lengeler, J. W. & Jacobson, G. R. Phosphoenolpyruvate:carbohydrate phosphotransferase systems of bacteria. *Microbiol. Rev.* **57**, 543–594 (1993).
21. Deutscher, J., Francke, C. & Postma, P. W. How phosphotransferase system-related protein phosphorylation regulates carbohydrate metabolism in bacteria. *Microbiol. Mol. Biol. Rev.* **70**, 939–1031 (2006).
A milestone in PTS reviews that discusses and summarizes everything that has been learned about the regulatory functions of the PTS since its discovery in 1964.
22. Cases, I., Velázquez, F. & de Lorenzo, V. The ancestral role of the phosphoenolpyruvate-carbohydrate phosphotransferase system (PTS) as exposed by comparative genomics. *Res. Microbiol.* **158**, 666–670 (2007).
23. Hogema, B. M. *et al.* Inducer exclusion in *Escherichia coli* by non-PTS substrates: the role of the PEP to pyruvate ratio in determining the phosphorylation state of enzyme IIA^{Glc}. *Mol. Microbiol.* **30**, 487–498 (1998).
The first demonstration that the PEP to pyruvate ratio is a major factor in the control of the phosphorylation state of $EIIA^{Glc}$ and therefore CCR in *E. coli*. The mechanism explains how the use of non-PTS carbon sources causes CCR.
24. Bettenbrock, K. *et al.* Correlation between growth rates, $EIIA^{Crr}$ phosphorylation, and intracellular cyclic AMP levels in *Escherichia coli* K-12. *J. Bacteriol.* **189**, 6891–6900 (2007).
25. Feucht, B. U. & Saier, M. H. Jr. Fine control of adenylate cyclase by the phosphoenolpyruvate:sugar phosphotransferase systems in *Escherichia coli* and *Salmonella typhimurium*. *J. Bacteriol.* **141**, 603–610 (1980).
26. Harwood, J. P. *et al.* Involvement of the glucose enzymes II of the sugar phosphotransferase system in the regulation of adenylate cyclase by glucose in *Escherichia coli*. *J. Biol. Chem.* **251**, 2462–2468 (1976).
27. Park, Y.-H., Lee, B. R., Seok, Y.-J. & Peterkofsky, A. *In vitro* reconstitution of catabolite repression in *Escherichia coli*. *J. Biol. Chem.* **281**, 6448–6454 (2006).
28. Malan, T. P., Kolb, A., Buc, H. & McClure, W. R. Mechanism of CRP–cAMP activation of *lac* operon transcription initiation activation of the P1 promoter. *J. Mol. Biol.* **180**, 881–909 (1984).
29. Tagami, H. & Aiba, H. A common role of CRP in transcription activation: CRP acts transiently to stimulate events leading to open complex formation at a diverse set of promoters. *EMBO J.* **17**, 1759–1767 (1998).
30. Busby, S. & Ebright, R. H. Transcription activation by catabolite activator protein (CAP). *J. Mol. Biol.* **293**, 199–213 (1999).
31. Papenfort, K. *et al.* Systematic deletion of *Salmonella* small RNA genes identifies CyaR, a conserved Crp-dependent riboregulator of OmpX synthesis. *Mol. Microbiol.* **68**, 890–906 (2008).
32. Polayes, D. A., Rice, P. W., Garner, M. M. & Dahlberg, J. E. Cyclic AMP — cyclic AMP receptor protein as a repressor of transcription of the *spf* gene of *Escherichia coli*. *J. Bacteriol.* **170**, 3110–3114 (1988).
33. Inada, T., Kimata, K & Aiba, H. Mechanism responsible for glucose–lactose diauxie in *Escherichia coli*: challenge to the cAMP model. *Genes Cells* **1**, 293–301 (1996).
A challenge to the text-book model of *lac* operon regulation. In a series of elegant experiments, this study shows how the CCR of the *lac* operon is caused by inducer exclusion and not by the cAMP–CRP pathway.
34. Hogema, B. M., Arents, J. C., Bader, R. & Postma, P. W. Autoregulation of lactose uptake through the LacY permease by enzyme IIA^{Glc} of the PTS in *Escherichia coli* K-12. *Mol. Microbiol.* **31**, 1825–1833 (1999).
35. Kimata, K., Takahashi, H., Inada, T., Postma, P. & Aiba, H. cAMP receptor protein–cAMP plays a crucial role in glucose–lactose diauxie by activating the major glucose transporter gene in *Escherichia coli*. *Proc. Natl Acad. Sci. USA* **94**, 12914–12919 (1997).
36. Warner, J. B. & Lolkema, J. S. CcpA-dependent carbon catabolite repression in bacteria. *Microbiol. Mol. Biol. Rev.* **67**, 475–490 (2003).
37. Henkin, T. M., Grundy, F. J., Nicholson, W. L. & Chambliss, G. H. Catabolite repression of α-amylase gene expression in *Bacillus subtilis* involves a trans-acting gene product homologous to the *Escherichia coli lacI* and *galR* repressors. *Mol. Microbiol.* **5**, 575–584 (1991).
The discovery of CcpA in *B. subtilis*. Used transposon mutagenesis to screen for mutations that lead to the loss of glucose repression of the *amyE* gene and identified the *ccpA* gene.
38. Deutscher, J., Küster, E., Bergstedt, U., Charrier, V. & Hillen, W. Protein kinase-dependent HPr/CcpA interaction links glycolytic activity to carbon catabolite repression in Gram-positive bacteria. *Mol. Microbiol.* **15**, 1049–1053 (1995).
Demonstrates for the first time that HPr(Ser-P) specifically interacts with CcpA in the presence of fructose-1,6-bisphosphate, which suggests that HPr(Ser-P) is a cofactor for CcpA activity.
39. Jones, B. E. *et al.* Binding of the catabolite repressor protein CcpA to its DNA target is regulated by phosphorylation of its corepressor HPr. *J. Biol. Chem.* **272**, 26530–26535 (1997).
40. Nessler, S. *et al.* HPr kinase/phosphorylase, the sensor enzyme of catabolite repression in Gram-positive bacteria: structural aspects of the enzyme and the complex with its protein substrate. *J. Bacteriol.* **185**, 4003–4010 (2003).
41. Galinier, A. *et al.* New protein kinase and protein phosphatase families mediate signal transduction in bacterial catabolite repression. *Proc. Natl Acad. Sci. USA* **95**, 1823–1828 (1998).
42. Reizer, J. *et al.* A novel bacterial protein kinase that controls carbon catabolite repression. *Mol. Microbiol.* **27**, 1157–1169 (1998).
43. Jault, J.-M. *et al.* The HPr kinase from *Bacillus subtilis* is a homo-oligomeric enzyme which exhibits strong positive cooperativity for nucleotide and fructose 1,6-bisphosphate binding. *J. Biol. Chem.* **275**, 1773–1780 (2000).
44. Mijakovic, I. *et al.* Pyrophosphate-producing protein dephosphorylation by HPr kinase/phosphorylase: a relic of early life? *Proc. Natl Acad. Sci. USA* **99**, 13442–13447 (2002).
45. Schumacher, M. A. *et al.* Structural basis for allosteric control of the transcription regulator CcpA by the phosphoprotein HPr-Ser46-P. *Cell* **118**, 731–741 (2004).
Reports the structure of the ternary complex of CcpA and HPr(Ser-P) bound to its DNA target. A comparison with the structure of Apo–CcpA reveals the structural changes that occur upon the binding of HPr(Ser-P) in CcpA and that favour DNA-binding of the complex.
46. Reizer, J. *et al.* Catabolite repression resistance of *gnt* operon expression in *Bacillus subtilis* conferred by mutation of His-15, the site of phosphoenolpyruvate-dependent phosphorylation of the phosphocarrier protein HPr. *J. Bacteriol.* **178**, 5480–5486 (1996).
47. Seidel, G., Diel, M., Fuchsbauer, N. & Hillen, W. Quantitative interdependence of coeffectors, CcpA and *cre* in carbon catabolite regulation of *Bacillus subtilis*. *FEBS J.* **272**, 2566–2577 (2005).
48. Schumacher, M. A., Seidel, G., Hillen, W. & Brennan, R. G. Structural mechanism for the fine-tuning of CcpA function by the small molecule effectors glucose 6-phosphate and fructose 1,6-bisphosphate. *J. Mol. Biol.* **368**, 1042–1050 (2007).
49. Galinier, A. *et al.* The *Bacillus subtilis crh* gene encodes a HPr-like protein involved in carbon catabolite repression. *Proc. Natl Acad. Sci. USA* **94**, 8439–8444 (1997).
50. Martin-Verstraete, I., Deutscher, J. & Galinier, A. Phosphorylation of HPr and Crh by HPrK, early steps in the catabolite repression signalling pathway for the *Bacillus subtilis* levanase operon. *J. Bacteriol.* **181**, 2966–2969 (1999).
51. Galinier, A., Deutscher, J. & Martin-Verstraete, I. Phosphorylation of either Crh or HPr mediates binding of CcpA to the *Bacillus subtilis xyn cre* and catabolite repression of the *xyn* operon. *J. Mol. Biol.* **286**, 307–314 (1999).
52. Görke, B., Fraysse, L. & Galinier, A. Drastic differences in Crh and HPr synthesis levels reflect their different impacts on catabolite repression in *Bacillus subtilis*. *J. Bacteriol.* **186**, 2992–2995 (2004).
53. Schumacher, M. A., Seidel, G., Hillen, W. & Brennan, R. G. Phosphoprotein Crh-Ser46-P displays altered binding to CcpA to effect carbon catabolite regulation. *J. Biol. Chem.* **281**, 6793–6800 (2006).
54. Miwa, Y., Nakata, A., Ogiwara, A., Yamamoto, M. & Fujita, Y. Evaluation and characterization of catabolite-responsive elements (*cre*) of *Bacillus subtilis*. *Nucleic Acids Res.* **28**, 1206–1210 (2000).
55. Nicholson, W. L. *et al.* Catabolite repression-resistant mutations of the *Bacillus subtilis* alpha-amylase promoter affect transcription levels and are in an operator-like sequence. *J. Mol. Biol.* **198**, 609–618 (1987).
56. Inacio, J. M. & de Sá-Nogueira, I. *trans*-acting factors and *cis*-elements involved in glucose repression of arabinan degradation in *Bacillus subtilis*. *J. Bacteriol.* **189**, 8371–8376 (2007).
57. Choi, S. K. & Saier, M. H. Jr. Regulation of *sigL* expression by the catabolite control protein CcpA involves a roadblock mechanism in *Bacillus subtilis*: potential connection between carbon and nitrogen metabolism. *J. Bacteriol.* **187**, 6856–6861 (2005).
58. Grundy, F. J., Waters, D. A., Allen, S. H. & Henkin, T. M. Regulation of the *Bacillus subtilis* acetate kinase gene by CcpA. *J. Bacteriol.* **175**, 7348–7355 (1993).
59. Presecan-Siedel, E. *et al.* The catabolite regulation of the *pta* gene as part of carbon flow pathways in *Bacillus subtilis*. *J. Bacteriol.* **181**, 6889–6897 (1999).
60. Ludwig, H., Rebhan, N., Blencke, H.-M., Merzbacher, M. & Stülke, J. Control of the glycolytic *gapA* operon by the catabolite control protein A in *Bacillus subtilis*: a novel mechanism of CcpA-mediated regulation. *Mol. Microbiol.* **45**, 543–553 (2002).
61. Renna, N. C., Najimudin, N., Winik, L. R. & Zahler, S. A. Regulation of the *Bacillus subtilis alsS*, *alsD*, and *alsR* genes involved in post-exponential production of acetoin. *J. Bacteriol.* **175**, 3863–3875 (1993).
62. Winkler, H. H. & Wilson, T. H. Inhibition of β-galactoside transport by substrates of the glucose transport system in *Escherichia coli*. *Biochim. Biophys. Acta* **135**, 1030–1051 (1967).
63. Nelson, S. O., Wright, J. K. & Postma, P. W. The mechanism of inducer exclusion. Direct interaction between purified III^{Glc} of the phosphoenolpyruvate: sugar phosphotransferase system and the lactose carrier of *Escherichia coli*. *EMBO J.* **2**, 715–720 (1983).
Provides the first proof that $EIIA^{Glc}$ elicits inducer inclusion of the *lac* operon by direct binding and inhibition of the lactose permease.

64. Smirnova, I. *et al.* Sugar binding induces an outward facing conformation of LacY. *Proc. Natl Acad. Sci. USA* **104**, 16504–16509 (2007).
65. Titgemeyer, F., Mason, R. E. & Saier, M. H. Jr. Regulation of the raffinose permease of *Escherichia coli* by the glucose-specific enzyme IIA of the phosphoenolpyruvate:sugar phosphotransferase system. *J. Bacteriol.* **176**, 543–546 (1994).
66. Misko, T. P., Mitchell, W. J., Meadow, N. D. & Roseman, S. Sugar transport by the bacterial phosphotransferase system. Reconstitution of inducer exclusion in *Salmonella typhimurium* membrane vesicles. *J. Biol. Chem.* **262**, 16261–16266 (1987).
67. Saier, M. H. Jr, Novotny, M. J., Comeau-Fuhrman, D., Osumi, T. & Desai, J. D. Cooperative binding of the sugar substrates and allosteric regulatory protein (enzyme III^{Glc} of the phosphotransferase system) to the lactose and melibiose permeases in *Escherichia coli* and *Salmonella typhimurium*. *J. Bacteriol.* **155**, 1351–1357 (1983).
68. Djordjevic, G. M., Tchieu, J. H. & Saier, M. H. Jr. Genes involved in control of galactose uptake in *Lactobacillus brevis* and reconstitution of the regulatory system in *Bacillus subtilis*. *J. Bacteriol.* **183**, 3224–3236 (2001).
69. Poolman, B., Knol, J., Mollet, B., Nieuwenhuis, B. & Sulter, G. Regulation of bacterial sugar-H^+ symport by phosphoenolpyruvate-dependent enzyme I/HPr-mediated phosphorylation. *Proc. Natl Acad. Sci. USA* **92**, 778–782 (1995).
70. Gunnewijk, M. G. & Poolman, B. Phosphorylation state of HPr determines the level of expression and the extent of phosphorylation of the lactose transport protein of *Streptococcus thermophilus*. *J. Biol. Chem.* **275**, 34073–34079 (2000).
71. Stülke, J., Arnaud, M., Rapoport, G. & Martin-Verstraete, I. PRD — a protein domain involved in PTS-dependent induction and carbon catabolite repression of catabolic operons in bacteria. *Mol. Microbiol.* **28**, 865–874 (1998).
72. Tortosa, P. *et al.* Sites of positive and negative regulation in the *Bacillus subtilis* antiterminators LicT and SacY. *Mol. Microbiol.* **41**, 1381–1393 (2001).
73. Krüger, S., Gertz, S. & Hecker, M. Transcriptional analysis of *bglPH* expression in *Bacillus subtilis*: evidence for two distinct pathways mediating carbon catabolite repression. *J. Bacteriol.* **178**, 2637–2644 (1996).
Demonstrates that CcpA and the antiterminator LicT provide independent mechanisms of CCR of the *B. subtilis* β-glucoside utilization system.
74. Lindner, C., Galinier, A., Hecker, M. & Deutscher, J. Regulation of the activity of the *Bacillus subtilis* antiterminator LicT by multiple PEP-dependent, enzyme I- and HPr-catalysed phosphorylation. *Mol. Microbiol.* **31**, 995–1006 (1999).
75. Lindner, C., Hecker, M., Le Coq, D. & Deutscher, J. *Bacillus subtilis* mutant LicT antiterminators exhibiting enzyme I- and HPr-independent antitermination affect catabolite repression of the *bglPH* operon. *J. Bacteriol.* **184**, 4819–4828 (2002).
76. Görke, B. & Rak, B. Catabolite control of *Escherichia coli* regulatory protein BglG activity by antagonistically acting phosphorylations. *EMBO J.* **18**, 3370–3379 (1999).
77. Martin-Verstraete, I. *et al.* Antagonistic effects of dual PTS catalyzed phosphorylation on the *Bacillus subtilis* transcriptional activator LevR. *Mol. Microbiol.* **28**, 293–303 (1998).
78. Schmalisch, M., Bachem, S. & Stülke, J. Control of the *Bacillus subtilis* antiterminator protein GlcT by phosphorylation: elucidation of the phosphorylation chain leading to inactivation of GlcT. *J. Biol. Chem.* **278**, 51108–51115 (2003).
79. Zomer, A. L., Buist, G., Larsen, R., Kok, J. & Kuipers, O. P. Time-resolved determination of the CcpA regulon of *Lactococcus lactis* subsp. *cremoris* MG1363. *J. Bacteriol.* **189**, 1366–1381 (2007).
80. Luesink, E. J., van Herpen, R. E., Grossiord, B. P., Kuipers, O. P. & de Vos, W. M. Transcriptional activation of the glycolytic *las* operon and catabolite repression of the *gal* operon in *Lactococcus lactis* are mediated by the catabolite control protein CcpA. *Mol. Microbiol.* **30**, 789–798 (1998).
81. Abranches, J. *et al.* CcpA regulates central metabolism and virulence gene expression in *Streptococcus mutans*. *J. Bacteriol.* **190**, 2340–2349 (2008).
82. Doi, R. H. & Kosugi, A. Cellulosomes: plant-cell-wall-degrading enzyme complexes. *Nature Rev. Microbiol.* **2**, 541–551 (2004).
83. Stevenson, D. M. & Weimer, P. J. Expression of 17 genes in *Clostridium thermocellum* ATCC 27405 during fermentation of cellulose or cellobiose in continuous culture. *Appl. Environ. Microbiol.* **71**, 4672–4678 (2005).
84. Abdou, L. *et al.* Transcriptional regulation of the *Clostridium cellulolyticum* "*cip-cel*" operon: a complex mechanism involving a catabolite-responsive element. *J. Bacteriol.* **190**, 1499–1506 (2008).
85. van Wezel, G. P. *et al.* A new piece of an old jigsaw: glucose kinase is activated posttranslationally in a glucose transport-dependent manner in *Streptomyces coelicolor* A3(2). *J. Mol. Microbiol. Biotechnol.* **12**, 67–74 (2007).
86. Kwakman, J. H. J. M. & Postma, P. W. Glucose kinase has a regulatory role in carbon catabolite repression in *Streptomyces coelicolor*. *J. Bacteriol.* **176**, 2694–2698 (1994).
87. Angell, S., Lewis, C. G., Buttner, M. J. & Bibb, M. J. Glucose repression in *Streptomyces coelicolor* A3(2): a likely regulatory role for glucose kinase. *Mol. Gen. Genet.* **244**, 135–143 (1994).
88. Commichau, F. M. & Stülke, J. Trigger enzymes: bifunctional proteins active in metabolism and in controlling gene expression. *Mol. Microbiol.* **67**, 692–702 (2008).
89. van Wezel, G. P., White, J., Young, P., Postma, P. W. & Bibb, M. J. Substrate induction and glucose repression of maltose utilization by *Streptomyces coelicolor* A3(2) is controlled by *malR*, a member of the *lacI-galR* family of regulatory genes. *Mol. Microbiol.* **23**, 537–549 (1997).
90. Hindle, Z. & Smith, C. P. Substrate induction and catabolite repression of the *Streptomyces coelicolor* glycerol operon are mediated through the GylR protein. *Mol. Microbiol.* **12**, 737–745 (1994).
91. Arndt, A. & Eikmanns, B. J. The alcohol dehydrogenase gene *adhA* in *Corynebacterium glutamicum* is subject to carbon catabolite repression. *J. Bacteriol.* **189**, 7408–7416 (2007).
92. Gerstmeir, R., Cramer, A., Dangel, P., Schaffer, S. & Eikmanns, B. J. RamB, a novel transcriptional regulator involved in acetate metabolism of *Corynebacterium glutamicum*. *J. Bacteriol.* **186**, 2798–2809 (2004).
93. Cramer, A., Auchter, M., Frunzke, J., Bott, M. & Eikmanns, B. J. RamB, the transcriptional regulator of acetate metabolism in *Corynebacterium glutamicum*, is subject to regulation by RamA and RamB. *J. Bacteriol.* **189**, 1145–1149 (2007).
94. Müller, C., Petruschka, L., Cuypers, H., Burchhardt, G. & Herrmann, H. Carbon catabolite repression of phenol degradation in *Pseudomonas putida* is mediated by the inhibition of the activator protein PhlR. *J. Bacteriol.* **178**, 2030–2036 (1996).
95. Moreno, R., Ruiz-Manzano, A., Yuste, L. & Rojo, F. The *Pseudomonas putida* Crc global regulator is an RNA binding protein that inhibits translation of the AlkS transcriptional regulator. *Mol. Microbiol.* **64**, 665–675 (2007).
Reveals the molecular mechanism by which Crc exerts CCR in *P. putida*. Also shows that Crc prevents expression of a regulatory protein by binding and inhibiting the translation of its mRNA.
96. Moreno, R. & Rojo, F. The target for the *Pseudomonas putida* Crc global regulator at the benzoate degradation pathway is the BenR transcriptional regulator. *J. Bacteriol.* **190**, 1539–1545 (2008).
97. Iyer, R., Baliga, N. S. & Camilli, A. Catabolite control protein A (CcpA) contributes to virulence and regulation of sugar metabolism in *Streptococcus pneumoniae*. *J. Bacteriol.* **187**, 8340–8349 (2005).
98. Shelburne, S. A. *et al.* A direct link between carbohydrate utilization and virulence in the major human pathogen group A *Streptococcus*. *Proc. Natl Acad. Sci. USA* **105**, 1698–1703 (2008).
Detected a direct link between CcpA-mediated CCR and the expression of virulence factors in *S. pyogenes*. Also shows that *ccpA* mutants are strongly impaired in virulence.
99. Kinkel, T. L. & McIver, K. S. CcpA-mediated repression of streptolysin S expression and virulence. *Infect. Immun.* 19 May 2008 (doi:10.1128/IAI.00343–08).
100. Almengor, A. C., Kinkel, T. L., Day, S. J. & McIver, K. S. The catabolite control protein CcpA binds to P*mga* and influences expression of the virulence regulator Mga in the group A *Streptococcus*. *J. Bacteriol.* **189**, 8405–8416 (2007).
101. Hondorp, E. R. & McIver, K. S. The Mga virulence regulon: infection where the grass is greener. *Mol. Microbiol.* **66**, 1056–1065 (2007).
102. Zeng, L., Wen, Z. T. & Burne, R. A. A novel signal transduction system and feedback loop regulate fructan hydrolase gene expression in *Streptococcus mutans*. *Mol. Microbiol.* **62**, 187–200 (2006).
103. Mengaud, J. *et al.* Pleiotropic control of *Listeria monocytogenes* virulence factors by a gene that is autoregulated. *Mol. Microbiol.* **5**, 2273–2283 (1991).
104. Milenbachs, A. A., Brown, D. P., Moors, M. & Youngman, P. Carbon-source regulation of virulence gene expression in *Listeria monocytogenes*. *Mol. Microbiol.* **23**, 1075–1085 (1997).
105. Mertins, S. *et al.* Interference of components of the phosphoenolpyruvate phosphotransferase system with the central virulence gene regulator PrfA of *Listeria monocytogenes*. *J. Bacteriol.* **189**, 473–490 (2007).
106. Dupuy, B. & Sonenshein, A. L. Regulated transcription of *Clostridium difficile* toxin genes. *Mol. Microbiol.* **27**, 107–120 (1998).
107. Varga, J., Stirewalt, V. L. & Melville, S. B. The CcpA protein is necessary for efficient sporulation and enterotoxin gene (*cpe*) regulation in *Clostridium perfringens*. *J. Bacteriol.* **186**, 5221–5229 (2004).
108. Mendez, M. *et al.* Carbon catabolite repression of type IV pilus-dependent gliding motility in the anaerobic pathogen *Clostridium perfringens*. *J. Bacteriol.* **190**, 48–60 (2008).
109. Schmoll, T., Ott, M., Oudega, B. & Hacker, J. Use of a wild-type gene fusion to determine the influence of environmental conditions on expression of the S fimbrial adhesin in an *Escherichia coli* pathogen. *J. Bacteriol.* **172**, 5103–5111 (1990).
110. Nishikawa, Y., Scotland, S. M., Smith, H. R., Willshaw, G. A. & Rowe, B. Catabolite repression of the adhesion of Vero cytotoxin-producing *Escherichia coli* of serogroups O157 and O111. *Microb. Pathog.* **18**, 223–229 (1995).
111. Nagai, S., Yagihashi, T. & Ishihama, A. An avian pathogenic *Escherichia coli* strain produces a hemolysin, the expression of which is dependent on cyclic AMP receptor protein gene function. *Vet. Microbiol.* **60**, 227–238 (1998).
112. Dobrindt, U., Hochhut, B., Hentschel, U. & Hacker, J. Genomic islands in pathogenic and environmental microorganisms. *Nature Rev. Microbiol.* **2**, 414–424 (2004).
113. Teplitski, M., Goodier, R. I. & Ahmer, B. M. M. Catabolite repression of the SirA regulatory cascade in *Salmonella enterica*. *Int. J. Med. Microbiol.* **296**, 449–466 (2006).
114. Kelly, S. M., Bosecker, B. A. & Curtiss, R. Characterization and protective properties of attenuated mutants of *Salmonella choleraesuis*. *Infect. Immun.* **60**, 4881–4890 (1992).
115. Petersen, S. & Young, G. M. Essential role for cyclic AMP and its receptor protein in *Yersinia enterocolitica* virulence. *Infect. Immun.* **70**, 3665–3672 (2002).
116. Skorupski, K. & Taylor, R. K. Cyclic AMP and its receptor protein negatively regulate the coordinate expression of cholera toxin and toxin-coregulated pilus in *Vibrio cholerae*. *Proc. Natl Acad. Sci. USA* **94**, 265–270 (1997).
117. Kim, T.-J. *et al.* Direct transcriptional control of the plasminogen activator gene of *Yersinia pestis* by the cyclic AMP receptor protein. *J. Bacteriol.* **189**, 8890–8900 (2007).
118. Reverchon, S., Expert, D., Robert-Baudouy, J. & Nasser, W. The cyclic AMP receptor protein is the main activator of pectinolysis genes in *Erwinia chrysanthemi*. *J. Bacteriol.* **179**, 3500–3508 (1997).
119. De Lencastre, H. *et al.* Antibiotic resistance as a stress response: complete sequencing of a large number of chromosomal loci in *Staphylococcus aureus* strain COL that impact on the expression of resistance to methicillin. *Microb. Drug Resist.* **5**, 163–175 (1999).
120. Seidl, K. *et al. Staphylococcus aureus* CcpA affects virulence determinant production and antibiotic resistance. *Antimicrob. Agents Chemother.* **50**, 1183–1194 (2006).

121. Bizzini, A., Entenza, J. M. & Moreillon, P. Loss of penicillin tolerance by inactivating the carbon catabolite repression determinant CcpA in *Streptococcus gordonii*. *J. Antimicrob. Chemother.* **59**, 607–615 (2007).
122. Ramström, H. *et al.* Heterocylic bis-cations as starting hits for design of inhibitors of the bifunctional enzyme histidine-containing protein kinase/phosphatase from *Bacillus subtilis*. *J. Med. Chem.* **47**, 2264–2275 (2004).
123. Chu, C.-Y. *et al.* Heterologous protection in pigs induced by plasmid-cured and *crp* gene-deleted *Salmonella choleraesuis* live vaccine. *Vaccine* **25**, 7031–7040 (2007).
124. Curtiss, R. & Kelly, S. M. *Salmonella typhimurium* deletion mutants lacking adenylate cyclase and cyclic AMP receptor protein are avirulent and immunogenic. *Infect. Immun.* **55**, 3035–3043 (1987).
125. Chassagnole, C., Noisommit-Rizzi, N., Schmid, J. W., Mauch, K. & Reuss, M. Dynamic modeling of the central carbon metabolism of *Escherichia coli*. *Biotechnol. Bioeng.* **79**, 53–73 (2002).
126. Bettenbrock, K. *et al.* A quantitative approach to catabolite repression in *Escherichia coli*. *J. Biol. Chem.* **281**, 2578–2584 (2006).
A complete mathematical model of CCR of lactose and glycerol utilization in *E. coli* in which model predictions were experimentally verified.
127. Shen-Orr, S., Milo, R., Mangan, S. & Alon, U. Network motifs in the transcriptional regulation network of *Escherichia coli*. *Nature Genet.* **31**, 64–68 (2002).
128. van Tilbeurgh, H. & Declerck, N. Structural insights into the regulation of bacterial signalling proteins containing PRDs. *Curr. Opin. Struct. Biol.* **11**, 685–693 (2001).
129. Graille, M. *et al.* Activation of the LicT transcriptional antiterminator involves a domain swing/lock mechanism provoking massive structural changes. *J. Biol. Chem.* **280**, 14780–14789 (2005).

Acknowledgements
The authors acknowledge J. Deutscher and the members of their laboratories for helpful discussions. Work in the authors' laboratories is supported by grants from the German Research Foundation, the Fonds der Chemischen Industrie and the Ministry of Education and Research (BMBF; SysMO grant number PtJ-BIUO/0313978D).

DATABASES

Entrez Gene: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene
ackA | *cpe* | *mga* | *pilT* | *sfaA* | *sigL*
Entrez Genome Project: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=genomeprj
Bacillus subtilis | *Bifidobacterium longum* | *Chlamydia trachomatis* | *Clostridium cellulolyticum* | *Clostridium perfringens* | *Corynebacterium glutamicum* | *Erwinia chrysanthemi* | *Escherichia coli* | *Lactobacillus brevis* | *Lactococcus lactis* | *Listeria monocytogenes* | *Mycoplasma pneumoniae* | *Pseudomonas aeruginosa* | *Pseudomonas putida* | *Staphylococcus aureus* | *Streptococcus gordonii* | *Streptococcus thermophilus* | *Streptomyces coelicolor* | *Vibrio cholerae* | *Yersinia enterocolitica* | *Yersinia pestis*
Entrez Protein: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=protein
BglG | CAP | CcpA | Crh | CRP | Crr | HPrK | LevR | LicT | PrfA

FURTHER INFORMATION

Jörg Stülke's homepage: http://wwwuser.gwdg.de/~genmibio/stuelke.html
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