Carbohydrate utilization in bacteria:

Making the most out of sugars with the help of small regulatory RNAs

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ABSTRACT

Survival of bacteria in ever-changing habitats with fluctuating nutrient supplies requires rapid adaptation of their metabolic capabilities. To this end, carbohydrate metabolism is governed by complex regulatory networks including post-transcriptional mechanisms that involve small regulatory RNAs (sRNAs) and RNA-binding proteins. sRNAs limit the response to substrate availability, set the threshold or time required for induction and repression of carbohydrate utilization systems. Carbon catabolite repression (CCR) also involves sRNAs. In *Enterobacteriaceae*, sRNA Spot 42 cooperates with the transcriptional regulator cAMP-CRP to repress secondary carbohydrate utilization genes when a preferred sugar is consumed. In *Pseudomonads*, CCR operates entirely at the post-transcriptional level involving RNA-binding protein Hfq and decoy sRNA CrcZ. Moreover, sRNAs coordinate fluxes through central carbohydrate metabolic pathways with carbohydrate availability. In Gram-negative bacteria, the interplay between RNA-binding protein CsrA and its cognate sRNAs regulates glycolysis and gluconeogenesis in response to signals derived from metabolism. Spot 42 and cAMP-CRP jointly down-regulate tricarboxylic acid cycle activity when glycolytic carbon sources are ample. In addition, bacteria use sRNAs to reprogram carbohydrate metabolism in response to anaerobiosis and iron limitation. Finally, sRNAs also provide homeostasis of essential anabolic pathways as exemplified by the hexosamine pathway providing cell envelope precursors. In this review, we discuss the manifold roles of bacterial small RNAs for regulation of carbon source uptake and utilization, substrate prioritization and metabolism.

INTRODUCTION

Carbohydrates are degraded in central metabolic pathways namely glycolysis, the pentose phosphate pathway and the tricarboxylic acid (TCA) cycle to fuel cells with energy and building blocks to synthesize all biomolecules. A functional carbohydrate metabolism requires sufficient supply with carbon sources but also coordination with the availability of other nutrients and cellular activities. Hence, bacterial carbohydrate metabolism is controlled at all levels by large and densely interconnected regulatory networks (1). In recent years, post-transcriptional mechanisms involving small regulatory RNAs (sRNAs) have emerged as an additional layer in these networks. Extensive cross-talk of sRNAs with transcriptional regulators ensures a fine-tuned and coordinated metabolism.

Bacterial sRNAs come in two flavors: *cis*-encoded sRNAs are transcribed from the opposite strand of their target genes. Due to their perfect complementarity they form extensive RNA duplexes with their target transcripts influencing transcription, translation or degradation of the target (2). *Trans*-encoded sRNAs regulate distantly encoded targets that can either be RNA or protein. They regulate translation or RNA stability, either negatively or positively, through imperfect base-pairing (3). In addition, modulation of transcription termination by sRNAs has also been observed (4). In Gram-negative bacteria, *trans*-encoded sRNAs often require protein Hfq for protection from degradation and RNA duplex formation (5, 6). The activities of sRNAs are tightly controlled, either at the level of biogenesis or their decay (7-9). A recently emerging mechanism are decoy and sponge RNAs that are capable of sequestering sRNAs by base-pairing (10).

In this article, we review the manifold roles of sRNAs in regulation of carbohydrate metabolism. The outline of the review is illustrated in Fig. 1. First, specialized sRNAs that regulate consumption of particular carbohydrates are described (Fig. 1A). Bacteria residing in mixed environments often select the energetically most favorable carbon source. The regulatory contribution of sRNAs to substrate prioritization will be discussed subsequently (Fig. 1B). Next, we will dissect how transcriptional and post-transcriptional mechanisms cooperate to coordinate metabolic activities with carbohydrate availability and other cues such as iron and oxygen availability (Fig. 1C). Finally, we will discuss the amino sugar pathway generating precursors for cell envelope synthesis as an exemplary anabolic pathway regulated by sRNAs (Fig. 1D). To date, sRNAs are most thoroughly investigated in the Gram negative model bacteria *Escherichia coli* and *Salmonella*, but knowledge from unrelated species has increased and is incorporated.

SMALL RNAS REGULATING UTILIZATION OF PARTICULAR CARBON SOURCES

Heterotrophic bacteria such as *E. coli* and *Salmonella* can grow on a plethora of compounds as sole source of carbon and energy (11). To preserve resources, genes required for uptake and utilization of a particular carbon source are tightly regulated by substrate availability. Traditionally this task is thought to be achieved by dedicated transcription factors, for which the lactose repressor provides the classical paradigm (12). In fact, Jacob and Monod speculated that the Lac repressor could be an RNA acting at the post-transcriptional level (13), but this idea was largely forgotten until the first report of gene regulation by a small antisense RNA in bacteria (14). Meanwhile, several bacterial carbohydrate utilization systems are known to be fine-tuned

by sRNAs. The sRNAs in these circuits may limit the response to substrate availability, set the threshold concentration or modulate the delay time required for activation and shutdown of the system.

Post-transcriptional regulation of glucose uptake

Many bacteria including *E. coli* preferentially utilize glucose when growing in a mixture of carbon sources (15, 16), which also holds true for many enterobacterial pathogens when residing in mammalian host cells (17). In *E. coli*, glucose is internalized by the glucose transporter PtsG and to a minor degree by mannose transporter ManXYZ (18). Both transporters belong to the phosphotransferase system (PTS). PTS-transporters generate phosphosugars during transport. The phosphoryl-groups derived from phosphoenolpyruvate (PEP) are transferred via phospho-transferases enzyme I and HPr to the transporters including the $EIIA^{Glc}$ protein, which phosphorylates PtsG.

While phosphosugars are a primary energy source, high intracellular concentrations are toxic (19, 20). Such conditions cause rapid degradation of *ptsG* mRNA limiting further glucose uptake, which relieves stress (21, 22). The dedicated transcriptional regulator SgrR senses phosphosugar stress and induces expression of sRNA SgrS (23, 24). Hfq-assisted base-pairing of SgrS with *ptsG* inhibits translation and recruits endoribonuclease RNase E to degrade *ptsG* mRNA at the cytoplasmic membrane (23, 25-27). SgrT, a short peptide encoded by SgrS, contributes to stress relief by blocking glucose transport via direct inhibition of PtsG independently of SgrS base-pairing (28-30). In *E. coli*, SgrS regulates at least eight mRNAs by

direct base-pairing (31, 32). Downregulation of the *manXYZ* mRNA through a dual base-pairing mechanism prevents leaky glucose uptake (33, 34). Stabilization of the *yigL* mRNA, encoding a sugar phosphatase, by masking an RNase E cleavage site upon base-pairing allows export of sugars following their dephosphorylation (35-37).

While regulation of *manXYZ* and *yigL* clearly contributes to phosphosugar stress relief (36), the roles of the remaining targets *adiY*, *asd*, *folE*, *ptsI* and *purR* are less obvious (31, 32). The *ptsI* gene encodes enzyme I, which delivers phosphoryl-groups to all 21 PTS-transporters in *E. coli* (38, 39). Some of these transporters internalize sugars that generate glucose-6-phosphate or other phosphosugars upon catabolism. Thus, global deceleration of PTS activity may contribute to the phosphosugar stress response. Interestingly, phosphosugar stress elicited by a glucose analog or a block in glycolysis, e.g. by *pgi* mutation, can be rescued by addition of glycolytic intermediates downstream of the block (21, 40). This suggests that toxicity results from the depletion of a downstream metabolite, most likely PEP, rather than from accumulation of glucose-6-phosphate itself. This could explain the physiological roles of SgrS targets such as *asd*, which encodes an enzyme that converts aspartate to other amino acids. Downregulation of *asd* may preserve aspartate to replenish PEP and relieve stress (31).

The SgrS-mediated phosphosugar stress response seems conserved in *Enterobacteriaceae* and *Aeromonas* species (41, 42). However, PTS-type glucose transporters are much more widespread (18, 43). Do these bacteria also encounter phosphosugar stress and how do they cope? Downregulation of the *ptsG* transcript by glucose in a *pgi* mutant has also been observed for the Gram-positive *Corynebacterium glutamicum* (44). *C. glutamicum* lacks Hfq and therefore the underlying mechanism must differ from *E. coli*. A phosphosugar stress

response has not been reported for any of the Gram-positive *Firmicutes* species. However, as demonstrated for *Bacillus subtilis*, these bacteria may activate a glycolytic bypass, the methylglyoxal pathway, to prevent deleterious accumulation of phosphosugars (45).

Regulation of chitin and chitosugar utilization by sRNAs

Chitin is one of the most abundant polysaccharides on earth and particularly ample in aquatic environments representing an important carbon source for aquatic bacteria such as *Vibrionales*. In *Vibrio cholerae*, an important facultative human pathogen, chitin even serves as signal for natural competence. Chitin is sensed by the the orphan sensor kinase ChiS, which activates expression of chitin utilization genes by a still unknown mechanism (46, 47). ChiS further activates transcription factor TfoS, which is necessary for expression of the Hfq-dependent sRNA TfoR. This sRNA stimulates translation of TfoX, a regulator required for induction of competence (48, 49). In addition, TfoX induces expression of type VI secretion systems for killing of non-immune cells and subsequent acquisition of the released DNA (50). This mechanism provides a mechanistic basis for the high degree of genomic diversity observed in *V. cholerae*.

For *E. coli* and *Salmonella* chitin-derived carbohydrates represent a secondary carbon source as they become sporadically available as part of the hosts' diet. These species rely on excreted chitinases of other bacteria to convert chitin to chitosugars. Multiple transcriptional regulators and the sRNA ChiX are employed to restrict expression of chitosugar utilization genes to conditions of substrate sufficiency (51-53). Chitoporin ChiP required for uptake of chitosugars across the outer membrane is encoded in the *chiPQ* operon. ChiX inhibits *chiP* translation

initiation by base-pairing with its 5' UTR (54, 55) but also represses the distal cistron *chiQ* by facilitating Rho-dependent transcriptional termination (56). Interestingly, ChiX is not co-degraded with its target *chiP* but with a decoy RNA derived from the *chb* operon (54, 57). The *chb* operon encodes a PTS-transporter and enzymes for chitosugar uptake and degradation. Expression of the *chb* operon is activated by the operon-specific transcription regulator ChbR in response to chitosugar availability. When *chb* transcription rates are sufficiently high, base-pairing with the *chb* RNA-trap sequesters ChiX and relieves *chiPQ* repression boosting synthesis of chitoporin ChiP. Thereby, ChiX likely sets the delay time and threshold concentration for chitosugar utilization. ChiX only affects the *chb* transcript under non-inducing conditions leading to efficient silencing of the mRNA (53).

In the chitinolytic bacterium *Serratia marcescens*, ChiX coordinates synthesis of ChiP and chitin-degrading chitinases (58). Whereas *chiP*/ChiX base-pairing is conserved, the ChiX target site within the *chb* mRNA is lacking. In contrast, ChiX represses *chiR*, encoding a transcriptional activator of chitinase genes. Upon induction of *chiP* expression, ChiX is sequestered by base-pairing and repression of *chiR* is relieved (58). Thereby, ChiX couples induction of degrading enzymes to the expression of the specific transporters, coordinating extracellular breakdown of chitin with uptake of the products.

Regulation of mannitol uptake by a *cis*-encoded sRNA in *Vibrio cholerae*

In addition to chitin, mannitol represents an important carbon source for *V. cholerae* as it is produced in large quantities by marine algae. In *V. cholerae* synthesis of the mannitol PTS-

transporter MtlA is controlled by the *cis*-encoded sRNA MtlS through an Hfq-independent mechanism (59). MtlS is transcribed antisense to the mannitol *mtlADR* operon and shares 71 nt of perfect complementarity with the 5' UTR of *mtlA*. MtlS and *mtlA* form a stable duplex inhibiting *mtlA* translation without impairing transcript stability. How this affects the co-transcribed *mtlDR* genes is unknown. Close proximity of the *mtlA* and *mtlS* loci is required to efficiently repress *mtlA*, presumably by enabling rapid formation of the RNA duplex (60). Mannitol represses *mtlS* transcription, but the responsible regulator has not been identified. The MtlR repressor protein, which is encoded in the *mtlADR* operon itself, and MtlS appear to operate independently from each other (59, 61). MtlA was shown to activate biofilm formation suggesting that mannitol serves as extracellular signal for *V. cholerae* to colonize beneficial habitats (62). Mannitol may also act as compatible solute helping *V. cholerae* to withstand the high osmolarity in the human intestine (59). How these additional roles are integrated into the mannitol operon remains to be addressed.

Regulation of polysaccharide utilization genes by *cis*-encoded sRNAs in *Bacteroides*

Gram-negative *Bacteroidetes* are a dominating phylum of the microbiota in the human colon (63) and specialized in utilizing a wide variety of dietary polysaccharides and glycans derived from the mucosa of the gut (64). To this end, *Bacteroides* carry a large number of polysaccharide utilization loci (PULs), each one dedicated to the uptake and utilization of a specific glycan or polysaccharide. Each PUL encodes its own protein regulators for substrate-

dependent induction of the locus. RNA-seq analysis of *B. fragilis* revealed that many of these PULs transcribe sRNAs from the opposite strand (65). The antisense RNAs seem to be conserved as they are also observed in other *Bacteroides*. Overexpression of such a sRNA, DonS in *B. fragilis*, triggers loss of the corresponding *pul* transcript, causing disability to utilize corresponding host glycans. DonS may target the cognate *pul* mRNA to degradation or act through transcriptional interference by RNA polymerase collision, as observed for other antisense RNAs (2). Regulation by DonS might become relevant when the concentration of the inducing substrate declines leading to an excess of constitutively produced DonS over the *pul* transcript. Interestingly, the PULs shown to include antisense sRNAs are all involved in the utilization of host-derived glycans (65). Species like *B. thetaiotaomicron* preferentially utilize dietary polysaccharides if available and consequently repress the PULs for glycan utilization (66). Hence, it is possible that the DonS-like sRNAs mediate substrate prioritization in *Bacteroides*.

CARBON CATABOLITE REPRESSION AT THE POST-TRANSCRIPTIONAL LEVEL

In mixed environments bacteria often selectively utilize the carbon source favoring fastest growth (15, 67). In *E. coli*, uptake of the preferred substrate glucose triggers de-phosphorylation of the PTS, which activates mechanisms that prevent uptake and utilization of less preferred carbon sources – collectively known as carbon catabolite repression (CCR)(43, 68). Accumulation of non-phosphorylated $EIIA^{Glc}$ inhibits uptake of less preferred carbon sources by inducer exclusion. It also impedes production of cAMP, thereby preventing activation of carbohydrate utilization genes by the global transcription regulator CRP. While these mechanisms are well studied, the involvement of post-transcriptional mechanisms in CCR emerged only recently. A

213 global omics study in *E. coli* found over 90 genes to be post-transcriptionally regulated by CCR
214 (69). In the evolutionary distant *Pseudomonads*, CCR even appears to operate solely at the post-
215 transcriptional level (70).

216

217 Spot 42 – the third pillar of CCR in *Enterobacteriaceae*

218 The sRNA Spot 42 cooperates with cAMP-CRP in coherent feedforward loops to regulate
219 multiple carbohydrate metabolic genes (Fig. 2A) (71). It might therefore be considered the third
220 pillar of CCR, working in addition to the well-established mechanisms involving inducer
221 exclusion and cAMP (43). Spot 42 encoded by *spf* is one of few genes that are repressed by
222 cAMP-CRP *in E. coli* (72, 73). The first Spot 42 target discovered was *galK* encoding galactokinase
223 for galactose utilization (74). In presence of glucose, Spot 42 accumulates and selectively down-
224 regulates GalK without affecting the other proteins encoded in the *galETKM* operon, which have
225 additional functions for synthesis of UDP-sugars (Fig. 3) (74). Subsequent work revealed that
226 Spot42 has a global role in carbohydrate metabolism (75, 76). To date, its validated regulon
227 contains 29 genes (Fig. 2B), but is expected to increase further as many additional potential Spot
228 42 targets were identified by RIL-seq (77). This novel methodology identifies sRNA/mRNA pairs
229 by Hfq pull-down and subsequent ligation of bound RNAs. Impressively, RIL-seq recovered 11
230 previously validated Spot 42 targets emphasizing reliability of the method (Fig. 2B). Again, many
231 of the newly identified candidate targets have roles in carbohydrate metabolism (77).

232 Most targets are repressed by Spot 42 and where known, their transcription is activated
233 by cAMP-CRP fostering the hypothesis that Spot 42 cooperates with cAMP-CRP in coherent

feedforward loops to regulate carbohydrate utilization genes (Fig. 2)(75). In the presence of glucose, Spot 42 prevents leaky expression of these genes by targeting the few mRNAs produced despite inactivity of CRP. Furthermore, Spot 42 also shapes the dynamics of gene expression when cells shuttle between CCR and CCR-free conditions. Upon a shift to glucose-rich growth conditions, Spot 42 accelerates repression of the secondary carbohydrate utilization genes, which may facilitate adaptation to the more favorable growth condition. Vice versa, upon activation of CRP by cAMP, Spot 42 delays target activation, perhaps to prevent their premature activation in case glucose reappears (71, 75). Interestingly, many secondary carbon sources whose utilization is repressed by Spot 42 are available in the mucosa of mammalian guts (e.g. arabinose, N-acetylneuraminc acid, L-fucose; Fig. 2A), *E. coli*'s natural habitat (78), where Spot 42 may be particularly important for carbon source selection (71).

Until recently, cAMP-CRP was the only known regulator of Spot 42. However, RIL-seq identified a sponge sRNA, PspH, whose overexpression reduces Spot 42 levels and thus de-represses its targets (77). The role of this interaction is unknown. Another study reported induction of *spf* expression by pyruvate independent of cAMP-CRP (79) suggesting that the *spf* promoter is controlled by additional transcription factor(s) that remain to be identified.

Spot 42 base-pairing sites overlap or are close to the ribosomal binding site (RBS) (75, 76) and work on *galK* demonstrated that Spot 42 inhibits translation in an Hfq-dependent manner (74, 80). However, Spot 42 also alters target mRNA levels (75). In-depth study of the *galETKM* operon revealed that Spot 42 stimulates Rho-dependent transcription termination at the *galT-galK* junction re-capitulating observations for ChiX (56, 81). A non-canonical mechanism of Spot 42 action was observed for the *sdhCDAB* mRNA encoding succinate

dehydrogenase (82). Spot 42 pairs far upstream of the *sdhC* RBS and merely recruits Hfq, which inhibits translation. A direct role of Hfq as a translation repressor has also been reported for other mRNAs (83, 84), even in species beyond *E. coli* (70). Spot 42 contains three unstructured regions, each of them involved in target regulation explaining the high conservation of the entire Spot 42 sequence (75). In some cases, Spot 42 employs multiple base-pairing sites to regulate a single target, which might improve regulatory strength (76). Perhaps, multi-site pairing provides the flexibility required to regulate multiple targets, as observed for other sRNAs controlling exceptionally large regulons (77, 85, 86).

Outside of the *Enterobacteriales* the *spf* gene is found in four additional orders of γ-*Proteobacteria* including *Vibrionales* (87). In the latter order, the role of Spot 42 was also studied in the fish pathogen *Aliivibrio salmonicida* and in *V. parahaemolyticus*, which causes diarrhea and gastroenteritis in humans through consumption of contaminated seafood (88-90). In *A. samonicida spf* expression is negatively regulated by cAMP-CRP like in *E. coli* (88). Microarray analysis of a *spf* deletion mutant revealed up-regulation of genes involved in sugar catabolism, motility and chemotaxis (88). Thus, *A. salmonicida* Spot 42 may also impact on carbohydrate metabolism, but by targeting different genes than in *E. coli*, and carrying out additional roles. In *V. parahaemolyticus* Spot 42 is strongly upregulated during infection and impacts on the activities of two type III secretion systems (89, 90). It is tempting to speculate that Spot 42 coordinates the activities of the type III secretion systems with carbohydrate availability in the host.

CCR in *Pseudomonas* by Hfq-mediated translational repression

In *Pseudomonads*, CCR is regulated exclusively at the post-transcriptional level. Contrary to *E. coli*, *Pseudomonads* do not prefer glucose. Rather, succinate elicits the highest degree of CCR in *P. aeruginosa* but only weakly affects CCR in *P. putida* which prefers alkanes and branched-chain amino acids (91). CCR requires sRNA antagonists, the catabolite repression control protein Crc, and Hfq as master regulator. Hfq binds the 5' UTRs of mRNAs encoding transporters and catabolic enzymes for less-preferred carbon sources and directly blocks translation initiation (70). Protein Crc is required for efficient CCR and forms stable ternary complexes with Hfq and RNAs containing A-rich motifs (92, 93). Crc contributes to stability of these complexes through interaction with both Hfq and RNA (93).

Availability of the preferred carbon source coincides with low levels of sRNA antagonists for Hfq. *P. aeruginosa* possesses one such sRNA, CrcZ. *P. putida* encodes two, CrcY and CrcZ, and other species possess similar sRNAs (94-96). Expression of Crc-sRNAs is induced by the two-component system (TCS) CbrA/CbrB in absence of preferred carbon sources (92, 94-97). Kinase CbrA presumably senses internal stimuli reflecting the energetic state of the cell such as the α-ketoglutarate/glutamine ratio (91, 97). CrcZ of *P. aeruginosa* and CrcY/CrcZ of *P. fluorescens* bind Hfq with an approximately 5- to 20-fold higher affinity as compared to the mRNAs targeted by Hfq (70, 95, 98). Consequently, increased levels of the Crc sRNAs effectively sequester Hfq from its target transcripts relieving repression (92, 94). Through competition for Hfq, CrcZ may also interfere with riboregulation exerted by other sRNAs and thus indirectly impact their regulatory potential (98).

POST-TRANSCRIPTIONAL CONTROL OF CENTRAL CARBOHYDRATE METABOLISM

Post-transcriptional mechanisms coordinating central metabolism with carbohydrate availability

The activity of central carbohydrate metabolism is tightly coordinated with carbon supply by adjusting the amounts of corresponding enzymes in response to key metabolites, namely fructose-1,6-bisphosphate (FBP) and the PEP/pyruvate ratio (1, 99). In *E. coli*, FBP is sensed by the transcriptional regulator Cra, which represses glycolytic genes and activates genes involved in gluconeogenesis (100, 101). The PEP/pyruvate ratio determines the phosphorylation state of $EIIA^{Glc}$, which regulates adenylate cyclase and thus cAMP synthesis (102, 103). Importantly, FBP decreases the PEP/pyruvate ratio through feed-forward activation of pyruvate kinase and PEP carboxylase (1). A high FBP level activates glycolysis through inactivation of Cra and decreases activity of cAMP-CRP by inhibiting phosphorylation of $EIIA^{Glc}$. Of note, cAMP-CRP activates expression of TCA cycle enzymes (104), whose transcripts are repressed by Spot 42 (Fig. 3). Therefore, cAMP-CRP and Spot 42 also cooperate to re-direct metabolism from oxidative phosphorylation to fermentation when glycolytic carbon sources are available. Similar to CRP, Cra possesses a counterpart at the post-transcriptional level, which is the carbon storage regulatory Csr system.

Regulation of glycolysis and gluconeogenesis by the Csr system

Protein CsrA represents a global post-transcriptional regulator of diverse activities across bacterial species. In *E. coli* and other Gram-negative bacteria CsrA controls carbohydrate metabolic pathways, carbon source and nutrient acquisition, biofilm formation, motility, stress

responses and virulence (105, 106). CsrA binds mRNA substrates at GGA-motifs and mostly represses translation, but examples of positive regulation also exist (107). For instance, CsrA activates glycolysis by positively regulating mRNAs of several glycolytic enzymes, while repressing synthesis of enzymes for gluconeogenesis and the TCA cycle (Fig. 3; (106, 108, 109)). In fact, CsrA is essential for growth on glycolytic substrates reflecting its crucial role for an undisturbed carbohydrate metabolism (110). Flux analysis showed that stabilization of the *pfkA* mRNA encoding phosphofructokinase is crucial for regulation of glycolytic activity by CsrA (106, 109). CsrA also inhibits accumulation of the carbon storage compound glycogen and synthesis of the exopolysaccharide poly-β-1,6-N-acetyl-D-glucosamine (PGA), a major component of biofilm matrices (Fig. 3). Of note, CsrA was recently shown to bind Spot 42 and to activate target genes that are repressed by this sRNA (106). It remains to be shown whether CsrA binding inhibits Spot 42 base-pairing, thereby also influencing CCR. Further, binding to *cra* mRNA was also demonstrated but the physiological consequences are so far unclear. These sophisticated interconnections may expand the already complex regulatory network governing carbohydrate metabolism.

CsrA activity is antagonized by the decoy sRNAs CsrB and CsrC, which sequester CsrA by presenting multiple binding sites (111, 112). Transcription and decay of these sRNAs are controlled by signals derived from carbohydrate metabolism. The BarA/UvrY TCS activates transcription of both sRNAs in response to short chained carboxylic acids, e.g. acetate and formate, which accumulate when cells have expended glycolytic carbon sources and transition into stationary phase (Fig. 4; (113, 114)). Degradation of the sRNAs by RNase E requires the protein CsrD (115, 116). CsrD is activated by interaction with non-phosphorylated $EIIA^{Glc}$ in

presence of glycolytic substrates (102, 117). Together, activation of *csrB*/*csrC* transcription and slow-down of CsrB/CsrC decay increases abundance of these sRNAs when preferred carbon sources have been consumed (Fig. 4). The resulting shut-down of CsrA activity promotes the shift to stationary phase metabolism by repression of glycolytic genes and de-repression of gluconeogenetic and glycogen biosynthetic mRNAs (117, 118).

EIIA^{Glc} has an additional role for activity of the Csr system in *E. coli* as it also controls transcription of CsrB/CsrC through cAMP-CRP (119). cAMP-CRP represses *csrB* indirectly and *csrC* directly by blocking access of response regulator UvrY to the *csrC* promoter. Thus, non-phosphorylated EIIA^{Glc} has opposing effects as it activates the turnover but also transcription of these sRNAs (Fig. 4), creating an incoherent feed-forward loop with the potential to integrate further cues (119). For instance, the activity of adenylate cyclase is also inhibited by α-ketoglutarate signaling nitrogen limitation (Fig. 4) (120), which may affect CsrB/CsrC expression, but not degradation.

The Csr system is conserved in *Proteobacteria* albeit the number of CsrA paralogs and Csr sRNAs may vary (105). The regulatory links between Csr and EIIA^{Glc}/CRP may likewise differ as CsrD is absent in most *Proteobacteria* beyond the families *Enterobacteriaceae*, *Shewanellaceae* and *Vibrionaceae* (116). Similarly, control of *csrB*/*csrC* transcription may be different, e.g. in *Yersinia pseudotuberculosis*, a close relative of *E. coli*, expression of *csrC* is activated by PhoP/PhoQ rather than the BarA/UvrY TCS (121). In sum, the Csr system provides a further tier of controlling fluxes through central carbohydrate metabolic pathways in response to carbohydrate availability. In addition, CsrA may cross-talk to CCR and integrate information on the metabolic status into other intricately regulated processes, namely biofilm formation,

motility and pathogenicity. For more information on this topic the reader is referred to the chapter “Global regulation by CsrA and its RNA antagonists” in this article series.

Regulation of TCA cycle activity by small RNAs

ATP can either be produced by substrate-level phosphorylation or by oxidative phosphorylation. Respiration yields more ATP, but is also more costly as it requires more proteins. Bacteria sense the availability of carbon, oxygen and energy to efficiently regulate the TCA cycle and respiration. As already discussed, in *E. coli* the information on carbohydrate availability is integrated into the TCA cycle by CsrA, cAMP-CRP and sRNA Spot 42. sRNAs with comparable functions may also exist in unrelated bacteria. For instance, pathogenic *Neisseria* species employ two homologous sRNAs to repress transcripts of TCA cycle enzymes (122, 123). Overexpression of these sRNAs impairs growth of *N. menigitidis* in cerebrospinal fluid but not in blood, suggesting that they transfer information about the metabolic status to colonization of different niches in the host (122). In addition, activity of the TCA cycle is strongly shaped by availability of iron and oxygen. Again, sRNAs play prominent roles for these adaptations.

Downregulation of TCA cycle activity by small RNAs in response to iron limitation

Iron is indispensable for activity of numerous enzymes operating within major metabolic pathways. Upon limitation, bacteria redirect iron from non-essential to essential processes with the aid of transcription factor Fur and sRNA RyhB (124). Fur represses transcription of *ryhB* under iron sufficiency (125). However, upon iron starvation, RyhB is relieved from repression

and downregulates non-essential iron-containing proteins including TCA cycle enzymes (Fig. 3)(126) prompting cells to resort to fermentation (127). This trade-off enables essential pathways involving iron-dependent enzymes to remain functional when iron is scarce. Iron limitation in particular is encountered by pathogenic bacteria within the host (128). *Staphylococcus aureus* was shown to switch to fermentation inside the host, thereby producing lactate, which lowers the surrounding pH. This increases iron availability through release from host iron storage proteins (129). Switch to fermentation is restricted to bacteria that can grow anaerobically. In the obligate aerobe *Azotobacter vinelandii*, the functional analog of RyhB named ArrF does not affect TCA cycle-related enzymes, but rather represses genes involved in nitrogen fixation, a non-essential process (130).

Coordination of carbon metabolism with oxygen availability by sRNA FnrS in *Enterobacteriaceae*

Enterobacteriaceae are facultative anaerobes. In the absence of oxygen, *E. coli* uses alternative electron acceptors to procure anaerobic respiration. If oxygen is not available, NAD^+ is regenerated by fermenting carbon sources to mixed acids and ethanol (131). Two global transcription factors, ArcA and Fnr reprogram metabolism in response to anaerobiosis (132, 133). Fnr senses oxygen directly, whereas response regulator ArcA is activated by its cognate kinase ArcB when the redox state of the quinone pool changes. Upon anaerobiosis, Fnr and ArcA collectively activate genes of alternative electron transport chains and repress functions of aerobic metabolism including the TCA cycle, the glyoxylate shunt and respiratory NADH dehydrogenases (134-137).

Notably, Fnr and ArcA also employ sRNAs in their regulons. One of them, sRNA FnrS is conserved among *Enterobacteriaceae*. FnrS is only detectable in the absence of oxygen as its transcription strictly depends on Fnr and to a minor extent on ArcA (86, 138). Globally, FnrS appears to extend the regulons of Fnr and ArcA by acting as non-coding regulator to repress functions that are not required in absence of oxygen including enzymes of aerobic carbohydrate metabolism (Fig. 3)(86, 138). For other targets, e.g. *mqo* (Fig. 3), FnrS cooperates in coherent feed-forward loops as these genes are also directly repressed by Fnr or ArcA (86, 139). This also applies to *acnA* and *fumC*, but here FnrS acts indirectly through repression of MarA, which is a transcriptional activator of these TCA cycle genes – a regulatory scenario known as multistep coherent feed-forward loop (Fig. 3)(32, 138). RIL-seq revealed many additional metabolism-related transcripts putatively base-pairing with FnrS, including the *fnr* mRNA itself (77), hinting at a feed-back loop balancing Fnr and FnrS levels. FnrS is Hfq-dependent and appears to act primarily by inhibition of translation initiation (138). Interestingly, FnrS uses distinct sequences to base-pair with subsets of its targets. Transcripts linked to oxidative stress and folate metabolism appear to base-pair with the 5' end of FnrS, whereas mRNAs of central metabolic enzymes are regulated by a single-stranded region in the sRNA body (86). This functional specialization may reflect evolution of FnrS by fusion of two originally distinct sRNAs (86).

The *E. coli* and *Salmonella* ArcA regulon contains an additional sRNA, ArcZ, which is encoded downstream of the *arcB* gene and is only expressed under aerobic conditions. ArcZ limits accumulation of active ArcA through destabilization of the *arcB* mRNA (140) and targets further diverse functions, but is apparently not involved in regulation of carbohydrate metabolism (140-143). Recently, the sRNA EsrE was shown to activate synthesis of subunit SdhD

of succinate dehydrogenase in *E. coli* (Fig. 3) (144). EsrE somewhat appears as an aerobic opponent of FnrS as it is essential for aerobic growth on TCA cycle substrates, but the signal to which it responds remains unknown.

An sRNA activated in response to anaerobiosis was also identified in pathogenic *Neisseria* species (145, 146). These bacteria, which likely face oxygen limitation during host colonization, are capable of anaerobic respiration (147-149). The anaerobically induced sRNA was named AniS in *N. meningitidis* and FnrS in *N. gonorrhoeae* and both clearly belong to the Fnr regulon albeit sequence homology to enterobacterial FnrS is lacking (145, 146). So far, only few targets for these sRNAs are known and they do not contribute to a common metabolic process (146, 150), leaving it open whether these sRNAs are indeed functional equivalents of enterobacterial FnrS.

RsaE – a functional equivalent of FnrS in Gram-positive bacteria?

Small RNAs also play a role in regulation of central carbohydrate metabolism in Gram-positive *Firmicutes*. RsaE - later renamed RoxS in *B. subtilis* (151) - is besides the ubiquitous 6S RNA the sole *trans*-acting sRNA known to be conserved between staphylococci and *Bacillaceae* (152). Two independent studies linked RsaE of *S. aureus* to regulation of carbohydrate metabolism, amino acid transport and the folate pathway for one-carbon metabolism (152, 153). In particular, RsaE represses pyruvate dehydrogenase and several TCA cycle enzymes (152, 153). Consistently, downregulation of TCA cycle enzymes was also observed for RoxS in *B. subtilis* (151, 154).

Expression of RsaE/RoxS is induced by the response regulator ResD of the ResD/ResE TCS (151). *S. aureus* and *B. subtilis* are facultative anaerobes and can switch to fermentation or nitrate respiration in absence of oxygen. The ResD/ResE TCS (named SrrA/SrrB in *S. aureus*) responds to oxygen limitation or increased nitric oxide (NO) levels and activates genes required for anaerobic metabolism and NO detoxification (155). Nitrate respiration produces NO as by-product, which is likely sensed as an indicator of nitrate availability and leads to induction of RsaE/RoxS expression through ResD (SrrA) (151). Therefore, RoxS (RsaE) may extend the regulon of the ResD/ResE TCS, contributing to adaptation to anoxia. *B. subtilis* RoxS is additionally controlled by transcription factor Rex, which represses genes for fermentation under oxic conditions when the $NADH/NAD^+$ ratio is low (154). RoxS is transiently released from Rex repression when malate is utilized, which generates NADH in the early steps of catabolism (154). By stimulating synthesis of the malate transporter YflS, RoxS ensures continuous uptake of malate (154).

Detailed analysis of *yflS* regulation by RoxS revealed a novel mechanism how RNA degradation may be counteracted by sRNAs in Gram-positive bacteria. RoxS base-pairs with the 5’ end of the *yflS* mRNA thereby protecting it from RNase J1, which degrades RNA in 5’-3’ direction – an activity absent in *Enterobacteriaceae* (154). Among the negatively regulated RoxS targets, the *ppnKB* mRNA was studied in detail (151). Base-pairing inhibits translation but also creates an RNase III cleavage site destabilizing the mRNA. RoxS uses a C-rich motif for base-pairing - a feature shared by many Gram-positive sRNAs to prevent ribosome recruitment (156). RoxS is cleaved by endoribonuclease RNase Y. Intriguingly, processed and full-length RoxS

exhibit distinct regulatory potentials, albeit the physiological meaning of this functional specialization remains unclear (151).

Post-transcriptional regulation of anabolic carbohydrate pathways

A number of anabolic pathways using carbohydrates as substrates are regulated at the post-transcriptional level. One example is provided by CsrA, which regulates gluconeogenesis. Another important example is provided by the post-transcriptional control of biosynthesis of cell wall precursors, which must be safeguarded in growing cells, regardless of the nature of the carbon source and the catabolic pathway. In *Enterobacteriaceae* this task is achieved by two hierarchically acting sRNAs GlmY and GlmZ.

Regulation of the hexosamine pathway by sRNAs GlmY and GlmZ

Glucosamine-6-phosphate (GlcN6P) synthase GlmS catalyzes the first and rate-limiting step in the hexosamine biosynthesis pathway by converting fructose-6-phosphate to GlcN6P (Fig. 3), an essential precursor for cell wall and outer membrane biogenesis (157). Intracellular GlcN6P levels dictate the need for GlmS, whose amount is fine-tuned by post-transcriptional regulatory mechanisms. In Gram-positive bacteria, GlcN6P serves as co-factor for a ribozyme present in the 5' UTR of the *glmS* mRNA (158, 159). Following self-cleavage, the *glmS* mRNA is rapidly degraded by RNase J1 (160). In *Enterobacteriaceae* GlmS levels are feedback-regulated by two homologous sRNAs - GlmY and GlmZ (Fig. 5) (161, 162). Only GlmZ is a direct activator of *glmS* translation (163-165). When GlcN6P is plentiful, GlmZ is inactivated by RNase E cleavage,

493 which requires the dedicated adaptor protein RapZ (166). However, under GlcN6P depletion,
494 cleavage of GlmZ is counteracted to elevate GlmS amounts and replenish the GlcN6P pool. This
495 is achieved through sequestration of RapZ by the decoy sRNA GlmY, whose levels increase when
496 amounts of the metabolite decline (Fig. 5) (164, 166). Consequently, full-length GlmZ base-pairs
497 with the *glmS* leader and activates expression by disrupting an inhibitory stem-loop structure
498 thereby exposing the RBS (163, 165). GlmZ is an Hfq-dependent sRNA and a substrate of RNase
499 E, whereas GlmY is not recognized by either of the two proteins (166, 167).

500 RapZ represents a highly specialized RNA-binding protein as it exclusively binds GlmY and
501 GlmZ (Fig. 5) (166). Upon binding, no major structural rearrangements are observable in the
502 sRNAs suggesting that RapZ stimulates cleavage of GlmZ by RNase E through protein-protein
503 interaction (for a detailed discussion of RNase E, the reader is referred to the chapter “RNase E
504 and the high fidelity of orchestration of RNA metabolism” within this book). Recently, the crystal
505 structure of RapZ revealed an unusual quaternary structure comprising a domain swapped
506 dimer-of-dimers – an arrangement that is a prerequisite for RapZ activity *in vivo* (Fig. 5) (168).
507 The RNA-binding function is located in the C-terminus, which bears homology to a subdomain of
508 6-phosphofructokinase, implying that RapZ may have evolved through re-purposing of enzyme
509 components from central metabolism. Putative RNA-binding residues are surface exposed and
510 form basic patches around an extended loop. Intriguingly, a binding pocket for a non-protein
511 ligand is observed in close vicinity to the presumptive RNA-binding domain (168). It remains to
512 be seen whether this site binds GlcN6P, potentially interfering with sRNA binding. Identification
513 of the GlcN6P binding site may foster the rational design of artificial ligands that can be used for
514 antimicrobial chemotherapy (162).

CONCLUSION AND PERSPECTIVES

A decade ago, when a first review on the current topic was published, only a single target had been identified for SgrS and Spot 42, and together with GlmZ these were the only base-pairing sRNAs known to regulate carbohydrate metabolic genes (169). Meanwhile, such sRNAs are common and further examples are expected to follow. For instance, CCR in Gram-positive bacteria may also include post-transcriptional mechanisms, as two sRNAs are controlled by the CCR master regulator CcpA in *Streptococcus mutans* (170). Even though the contribution of post-transcriptional mechanisms to regulation of metabolism is evident, they are usually neglected in studies assessing metabolic flux control and carbon catabolite repression (171, 172). In addition, the regulatory mechanisms employed by sRNAs are much more diverse than previously envisioned. Novel principles include modulation of target accessibility to degrading RNases, regulation of Rho-dependent transcription termination, recruitment of Hfq as translational repressor and employment of decoy RNAs sequestering sRNAs or their interacting proteins.

RNAseq has pushed the development of sophisticated 'omics' approaches facilitating assessment of post-transcriptional regulators on a global scale. RIP-seq and CLIP-seq provide snapshots of substrates bound to RNA-binding proteins at a time and also identify RNA-binding sites (e.g. (173)). CLASH and RILseq enable the recovery of sRNA-mRNA duplexes revealing whole RNA networks in a single experiment (77, 174). Most recently, CLIP-seq, ribosome profiling, transcriptomics and proteomics are combined in 'multi-omics' approaches exploring several layers of regulation in parallel and genome-wide. Application of 'multi-omics' to *E. coli*

CsrA revealed novel targets and physiological roles but also confirmed the global character of this post-transcriptional regulator in coordinating bacterial lifestyles with metabolic cues (106). The additional integration of metabolic flux analyses could reveal the specific contribution of sRNAs such as Spot 42 to reprogramming of metabolism.

There is an intimate connection between metabolism and virulence for which carbohydrate-related sRNAs and their protein interaction partners play an important role (175, 176). In fact, bacterial pathogenesis can be regarded as a developmental program granting access to nutrients in a hostile host environment. For instance, mutants lacking CsrA are severely compromised in establishing an infection, which is not only a consequence of dysregulated metabolism, but may also result from dis-coordinated expression of virulence factors (177, 178). A recurrent theme observed in pathogenic bacteria is that sRNAs from the core genome are recruited to regulate horizontally acquired virulence functions, which also applies to SgrS, Spot 42 and GlmY/GlmZ (89, 90, 179, 180). In line with these observations, SgrS and GlmY are strongly upregulated in *Y. pseudotuberculosis* during infection (177). For more information on this interesting topic, the reader is referred to the chapter on sRNA functions for virulence in this article series.

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FIGURE LEGENDS

FIGURE 1 Manifold roles of sRNAs for regulation of carbohydrate metabolism in bacteria. Cartoon summarizing the major roles of small RNAs (depicted in red) for regulation of carbohydrate metabolism in bacteria. (**A)** Regulation of uptake and utilization of particular carbohydrates by sRNAs in various species. In *Enterobacteriaceae*, the *trans*-encoded sRNAs SgrS counteracts phosphosugar stress through repression of glucose transporters and activation of the sugar phosphatase YigL. sRNA ChiX down-regulates the chitosugar-specific porin ChiP, setting the threshold concentration for induction of degrading enzymes. Further examples include regulation of host glycan and mannitol uptake by *cis*-encoded sRNAs in *Bacteroides* and *Vibrio* species, respectively. **(B)** Role of sRNAs in CCR. In *Enterobacteriaceae* and *Vibrionales* the sRNA Spot 42 represses genes for utilization of secondary carbon sources. Spot 42 is repressed by cAMP-CRP and therefore only active in the presence of preferred sugars generating low cAMP levels. In *Pseudomonas* translation of mRNAs for utilization of secondary carbon sources is repressed by Hfq. In absence of preferred substrates the CbrA/CbrB TCS activates expression of the decoy sRNA CrcZ titrating Hfq from target transcripts. **(C)** sRNAs coordinate carbohydrate metabolism with carbohydrate, oxygen and iron availability. The RNA-binding protein CsrA activates glycolysis and represses gluconeogenesis by binding to corresponding RNAs. CsrA activity is counteracted through sequestration by sRNAs CsrB/CsrC whose levels are regulated by signals from metabolism. In the absence of oxygen, sRNAs such as FnrS in *E. coli* and RoxS in *B. subtilis* redirect metabolism from oxidative phosphorylation to anaerobic respiration or fermentation. Upon iron starvation sRNA RyhB represses TCA cycle enzymes to save iron for essential processes. **(D)** Example for an anabolic pathway regulated by sRNAs. In

580 *Enterobacteriaceae* two homologous sRNAs regulate the key enzyme GlmS to achieve
581 homeostasis of glucosamine-6-phosphate, an essential precursor for cell envelope synthesis.

582

583 **FIGURE 2** The transcriptional regulator cAMP-CRP and sRNA Spot 42 cooperate to trigger CCR in
584 *Enterobacteriaceae*. **(A)** CRP and Spot 42 participate in coherent feed-forward loops to prevent
585 utilization of the indicated secondary carbon sources when the preferred carbon source glucose
586 is present. In addition to cAMP-CRP, Spot 42 is regulated by base-pairing with the sponge RNA
587 PspH. **(B)** The validated Spot 42 regulon to date. Target genes that are also positively controlled
588 by cAMP-CRP at the level of transcription, are boxed. Microarray analysis of Spot 42 pulse
589 expression (75) and improved software prediction algorithms (32, 76) fostered the identification
590 of most targets. Additional targets were identified by human inference or by a CLIPseq approach
591 mapping Hfq binding sites on a global scale (74, 82, 173, 181). Several of these targets were
592 recovered by RIL-seq (77).

593

594 **FIGURE 3** Post-transcriptional regulation of central carbon metabolic pathways in *E. coli*. Effects
595 of small RNAs (depicted in red) and of the RNA-binding protein CsrA (blue) on synthesis of
596 enzymes involved in glycolysis, gluconeogenesis and the TCA cycle. A green asterisk and bold
597 letters indicate direct regulation by CsrA (106). Anabolic pathways directing synthesis of
598 glycogen, UDP-sugars and the biofilm compound PGA, are also shown.

599

FIGURE 4 Model of the interconnection of the CsrA system with central carbon metabolism. Decoy sRNAs CsrB and CsrC regulate CsrA activity by sequestering the protein from its target mRNAs. CsrA indirectly activates *csrB/csrC* transcription creating a negative feed-back loop. In fast growing cells, when CsrB/C levels are low, CsrA activates glycolytic genes and represses the TCA cycle, gluconeogenesis and glycogen synthesis. Metabolism of glycolytic carbon sources causes accumulation of FBP, which activates pyruvate kinase thereby reducing the PEP/pyruvate ratio. Intake of PTS-substrates and a low PEP/pyruvate ratio trigger dephosphorylation of $EIIA^{Glc}$ leading to activation of CsrD, which triggers degradation of CsrB/CsrC by RNase E. Upon accumulation of short carboxylic acids (R-COOH) as metabolic end products, expression of *csrB*/*csrC* is induced by the BarA/UvrY TCS. Deceleration of glycolytic activity elevates the PEP/pyruvate level and increases $EIIA^{Glc}$ phosphorylation leading to stabilization of CsrB/CsrC and titration of CsrA. $EIIA^{Glc}$~P stimulates adenylate cyclase CyaA, which converts ATP to cAMP. The cAMP-Crp complex inhibits transcription of *csrB/csrC*. Involvement of $EIIA^{Glc}$ in regulation of CsrB/CsrC synthesis as well as decay allows integration of further cues.

FIGURE 5 Role of RNase E adaptor protein RapZ in feedback regulation of glucosamine-6-phosphate synthase (GlmS) synthesis in *E. coli*. When GlcN6P is plentiful in the cell, RapZ prevents *glmS* up-regulation by targeting its activating sRNA GlmZ to cleavage by RNase E. Within the tripartite complex formed, the sRNA is envisioned to be sandwiched between the tetrameric RapZ and RNase E-NTD proteins (168). Processing results in functional inactivation of GlmZ and subsequent decline in GlmS levels. Conversely, under GlcN6P depletion, RapZ is predominantly sequestered in complexes with the homologous sRNA GlmY, whose levels

622 increase under this condition. Consequently, GlmZ remains in its active full-length form and
623 stimulates *glmS* expression. Higher levels of GlmS replenish GlcN6P levels in the cell. Whether
624 RapZ has an active role in sensing GlcN6P via direct binding of the metabolite within its CTD is
625 currently under investigation. The unusual tetrameric structure of RapZ is schematically
626 depicted in the box at the top, left. Each monomer is represented by one color and consists of
627 two globular domains, NTD and CTD, connected via flexible linkers. Three distinct surfaces
628 involved in self-interaction can be discerned: CTD-CTD, NTD-NTD as well as CTD-NTD (168).

629

630 ## REFERENCES

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FIGURE 1

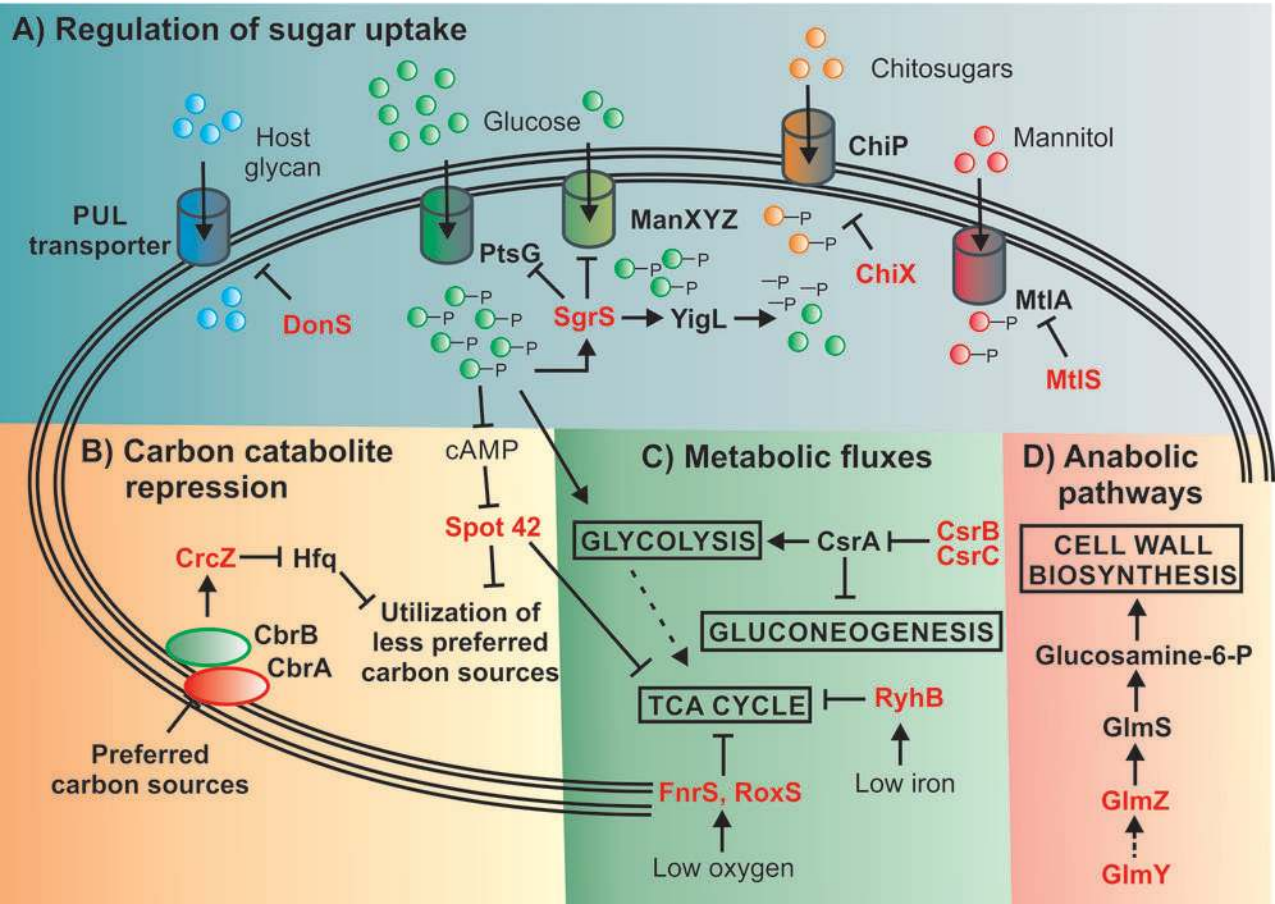

A) Regulation of sugar uptake
Chitosugars
Host glycan
Glucose
ChiP
Mannitol
PUL transporter
ManXYZ
PtsG
ChiX
DonS
SgrS
YigL
MtlA
MtlS
B) Carbon catabolite repression
cAMP
Spot 42
CrcZ
Hfq
Utilization of less preferred carbon sources
CbrB
CbrA
Preferred carbon sources
C) Metabolic fluxes
GLYCOLYSIS
CsrA
CsrB
CsrC
GLUCONEOGENESIS
TCA CYCLE
RyhB
Low iron
FnrS, RoxS
Low oxygen
D) Anabolic pathways
CELL WALL BIOSYNTHESIS
Glucosamine-6-P
GlmS
GlmZ
GlmY

FIGURE 2

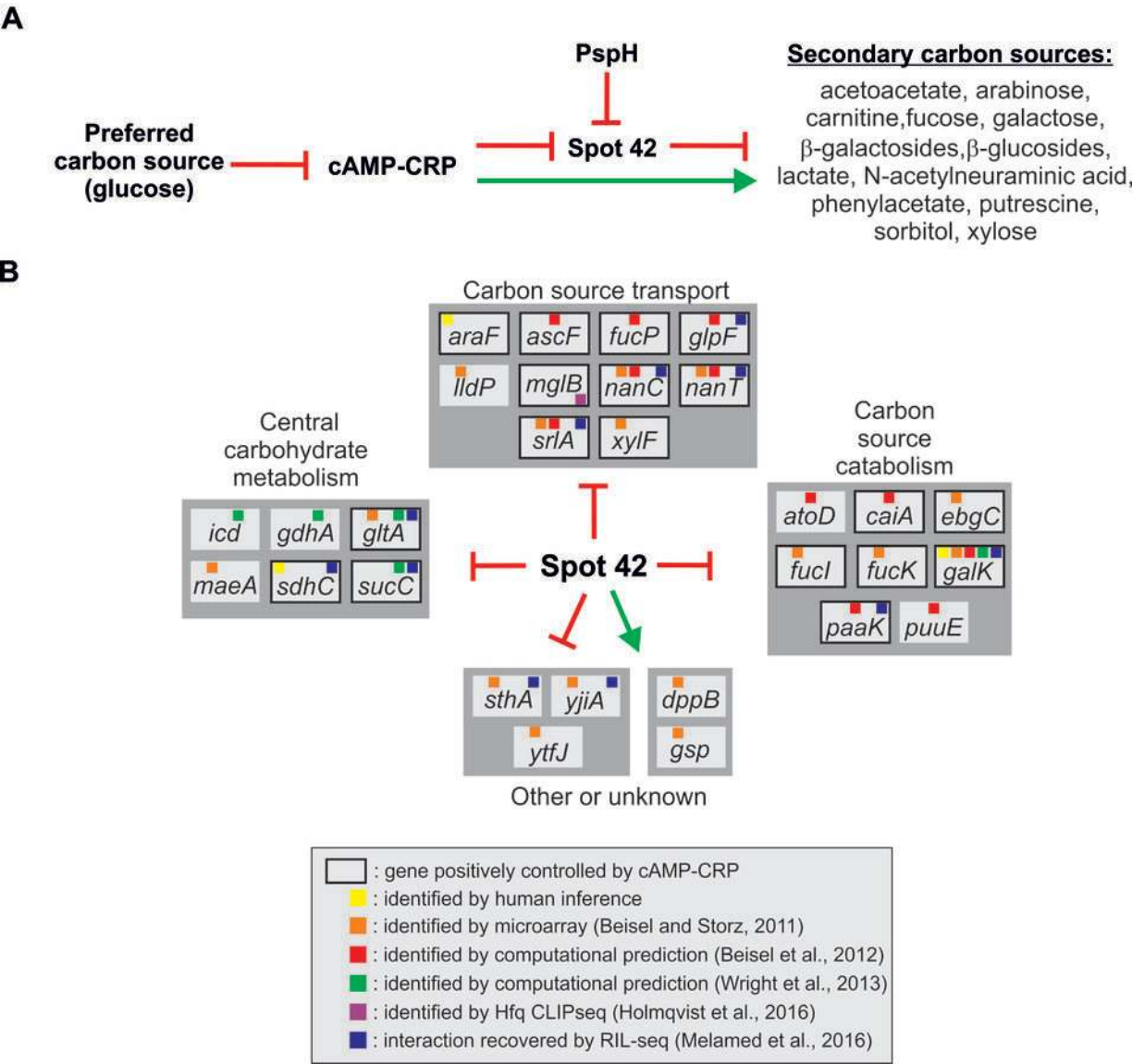

A
PspH
Secondary carbon sources:
acetoacetate, arabinose, carnitine,fucose, galactose, β-galactosides,β-glucosides, lactate, N-acetylneuraminic acid, phenylacetate, putrescine, sorbitol, xylose
Preferred carbon source (glucose)
cAMP-CRP
Spot 42
B
Carbon source transport
araF
ascF
fucP
glpF
lldP
mglB
nanC
nanT
srlA
xylF
Central carbohydrate metabolism
icd
gdhA
gltA
maeA
sdhC
sucC
Carbon source catabolism
atoD
caiA
ebgC
fucI
fucK
galK
paaK
puuE
Spot 42
sthA
yjiA
ytfJ
dppB
gsp
Other or unknown
: gene positively controlled by cAMP-CRP
: identified by human inference
: identified by microarray (Beisel and Storz, 2011)
: identified by computational prediction (Beisel et al., 2012)
: identified by computational prediction (Wright et al., 2013)
: identified by Hfq CLIPseq (Holmqvist et al., 2016)
: interaction recovered by RIL-seq (Melamed et al., 2016)

FIGURE 3

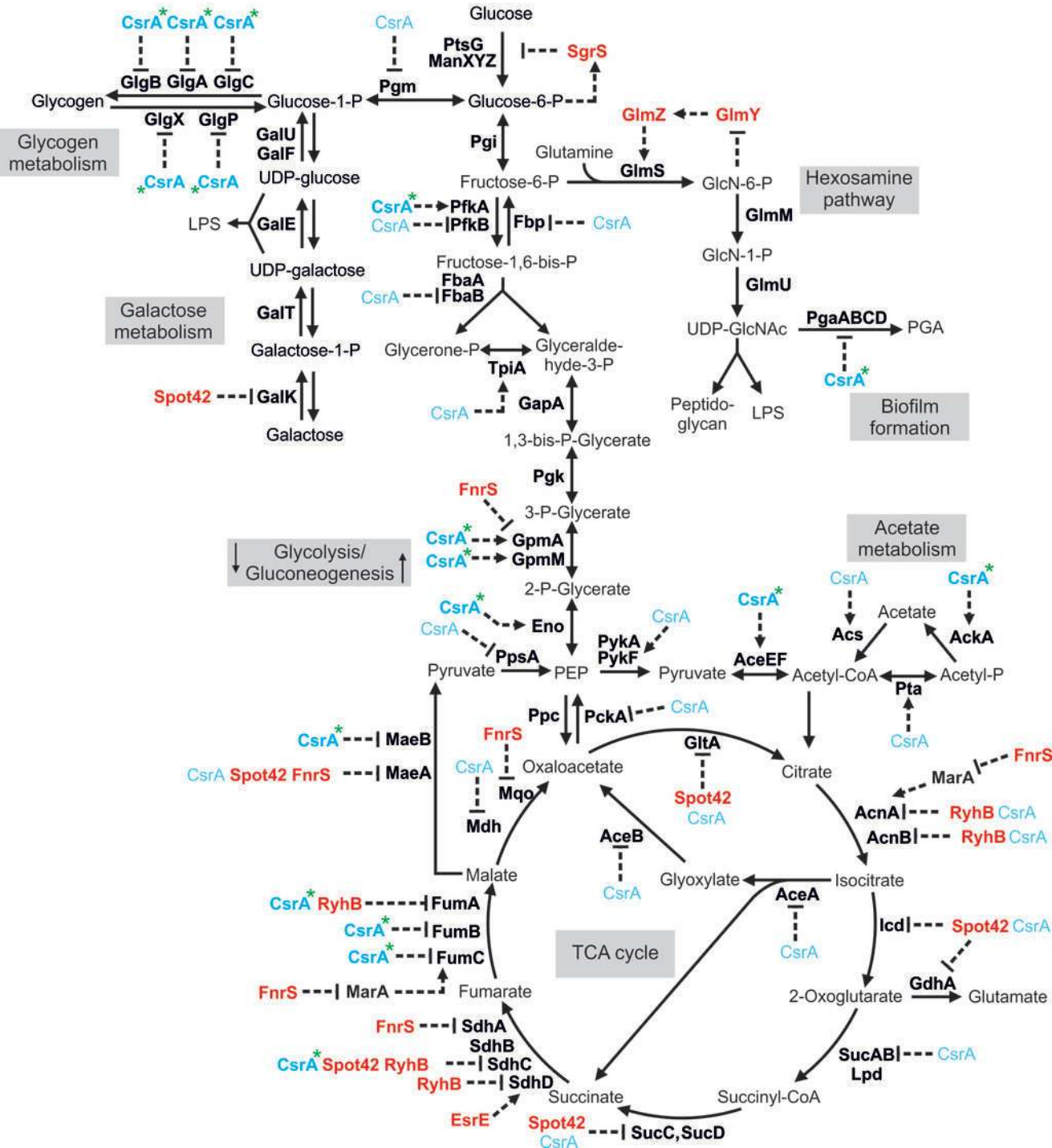

Glycogen metabolism
Galactose metabolism
Hexosamine pathway
Biofilm formation
Glycolysis/ Gluconeogenesis
Acetate metabolism
TCA cycle
Glycogen
Glucose-1-P
Glucose
Glucose-6-P
UDP-glucose
LPS
UDP-galactose
Galactose-1-P
Galactose
Fructose-6-P
Glutamine
GlcN-6-P
GlcN-1-P
UDP-GlcNAc
PGA
Peptido-glycan
LPS
Fructose-1,6-bis-P
Glycerone-P
Glyceralde-hyde-3-P
1,3-bis-P-Glycerate
3-P-Glycerate
2-P-Glycerate
PEP
Pyruvate
Pyruvate
Acetyl-CoA
Acetyl-P
Acetate
Oxaloacetate
Citrate
Isocitrate
2-Oxoglutarate
Glutamate
Succinyl-CoA
Succinate
Fumarate
Malate
Glyoxylate
CsrA
CsrA*
PtsG ManXYZ
SgrS
Pgm
GlgB GlgA GlgC
GlgX GlgP
GalU GalF
GalE
GalT
GalK
Spot42
Pgi
GlmS
GlmZ
GlmY
GlmM
GlmU
PgaABCD
PfkA
PfkB
Fbp
FbaA FbaB
TpiA
GapA
Pgk
FnrS
GpmA
GpmM
Eno
PpsA
PykA PykF
AceEF
Acs
AckA
Pta
Ppc
PckA
GltA
Mqo
Mdh
MaeB
MaeA
Spot42 FnrS
AceB
AceA
Icd
GdhA
MarA
AcnA
AcnB
RyhB
SucAB Lpd
FumA
FumB
FumC
SdhA SdhB SdhC SdhD
EsrE
SucC, SucD

FIGURE 4

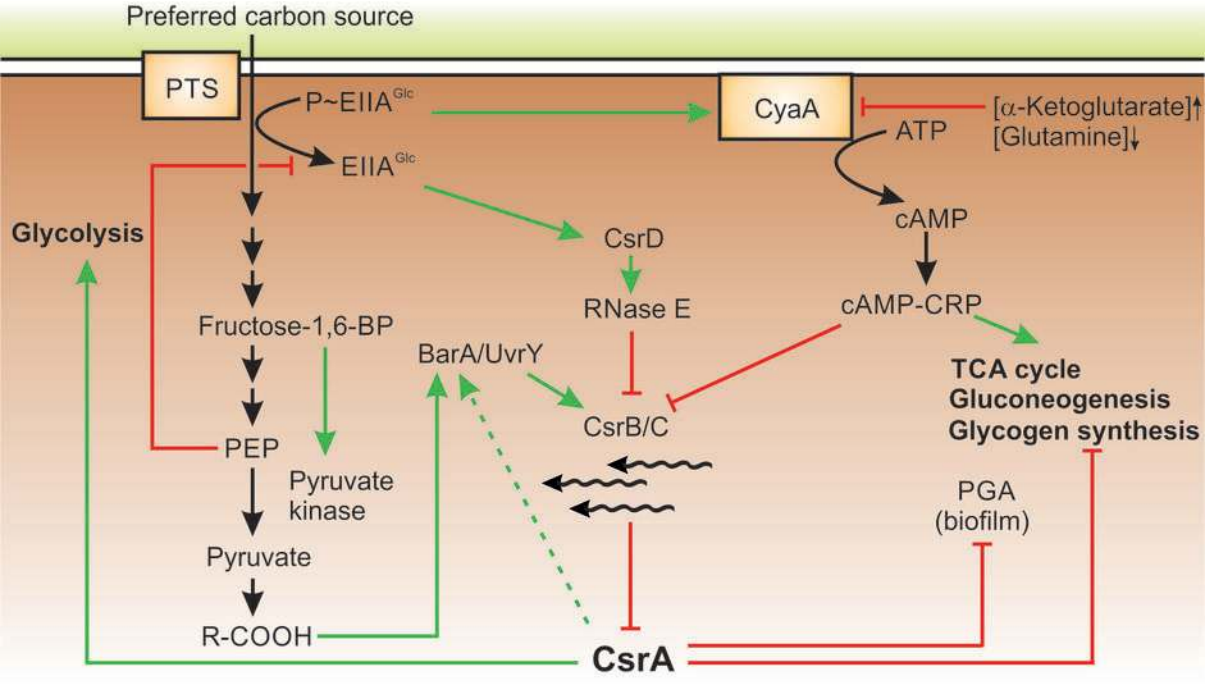
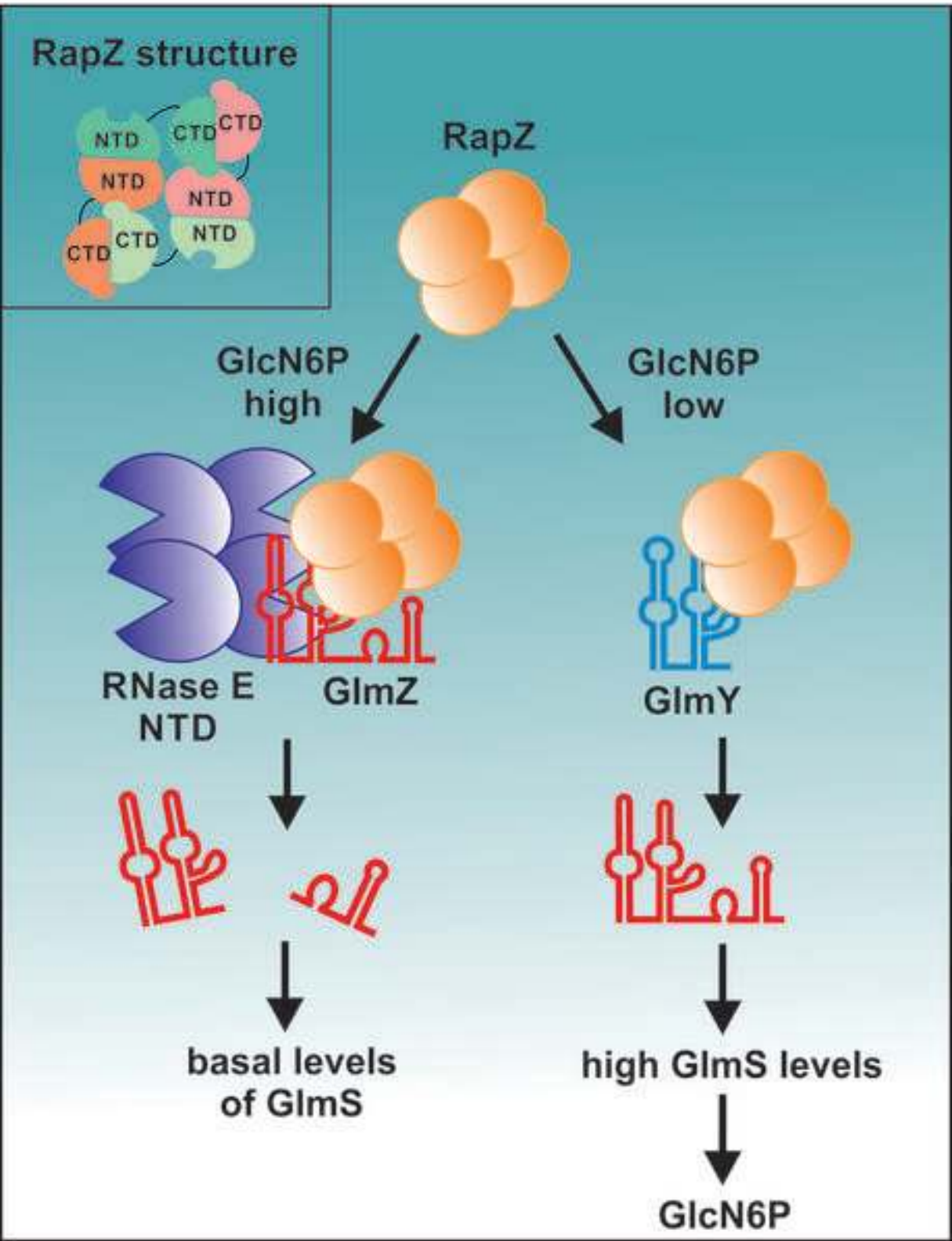

Preferred carbon source
PTS
P~EIIA^Glc
EIIA^Glc
CyaA
[α-Ketoglutarate]↑
[Glutamine]↓
ATP
cAMP
cAMP-CRP
Glycolysis
CsrD
RNase E
Fructose-1,6-BP
BarA/UvrY
CsrB/C
TCA cycle
Gluconeogenesis
Glycogen synthesis
PEP
Pyruvate kinase
PGA (biofilm)
Pyruvate
R-COOH
CsrA

FIGURE 5


RapZ structure
NTD
CTD
CTD
NTD
NTD
NTD
CTD
CTD
NTD
RapZ
GlcN6P high
GlcN6P low
RNase E NTD
GlmZ
GlmY
basal levels of GlmS
high GlmS levels
GlcN6P