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Award Review

Carbon Catabolite Control of the Metabolic Network in *Bacillus subtilis*

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The histidine-containing protein (HPr) is the energy coupling protein of the phosphoenolpyruvate-dependent carbohydrate:phosphotransferase system (PTS), which catalyzes the transport of carbohydrates in bacteria. In *Bacillus subtilis* and close relatives, global regulation of carbon catabolite control occurs on the binding of the complex of CcpA (catabolite control protein A) and P-Ser-HPr (seryl-phosphorylated form of HPr) to the catabolite responsive elements (*cre*) of the target operons, the constituent genes of which are roughly estimated to number 300. The complex of CcpA and P-Ser-HPr triggers the expression of several genes involved in the formation of acetate and acetoin, major extracellular products of *B. subtilis* grown on glucose. It also triggers the expression of an anabolic operon (*ilv-leu*) involved in the biosynthesis of branched-chain amino acids, which subsequently leads to cell propagation. On the other hand, this complex represses many genes and operons, which include an entrance gene for the TCA cycle (*citZ*), several transporter genes for TCA cycle-intermediates, some respiration genes, and many catabolic and anabolic genes involved in carbon, nitrogen, and phosphate metabolism, as well as for certain extracellular enzymes and secondary metabolites. Furthermore, these bacteria have CcpA-independent catabolite regulation systems, each of which involves a transcriptional repressor of CggR or CcpN. CggR and CcpN are derepressed under glycolytic and gluconeogenic growth conditions, and enhance glycolysis and gluconeogenesis respectively. Another CcpA-independent catabolite repression system involves P-His-HPr (histidyl-phosphorylated form of HPr). P-His-HPr phosphorylates and activates glycerol kinase, whose product is necessary for antitermination of the glycerol utilization operon through GlpP, the antiterminators (LicT and SacT, Y) of several operons for the utilization of less-preferred PTS-sugars, and some transcriptional activators such as LevR for the levan utilization operon. This phosphorylation is reduced due to the decreased level of P-His-HPr during active transport of a preferred PTS-carbohydrate such as glucose, resulting in catabolite repression of the target operons.

Thus CcpA-dependent and independent networks for carbon metabolism play a major role in the coordinate regulation of catabolism and anabolism to ensure optimum cell propagation in the presence and the absence of a preferred PTS-carbohydrate.

Key words: *Bacillus subtilis*; catabolite repression; catabolite activation; CcpA; HPr

Carbon catabolite control, *viz.*, carbon catabolite repression (CCR) and carbon catabolite activation (CCA), is a regulatory mechanism by which the cell coordinates the metabolism of carbon and energy sources to maximize its efficiency and regulates other metabolic processes as well. Also, carbon catabolite control is specific to carbon source-mediated regulation. CCR occurs when genes and operons are involved not only in catabolism but also in certain anabolic processes, such as the synthesis of certain extracellular enzymes and secondary metabolites that are not expressed as long as preferred sources of carbon and energy are present.[1] This can be achieved through transcription control of catabolic operons by global regulators in response to the availability of preferred carbon sources such as glucose, and/or by modulation of the intracellular availability of specific inducers of catabolic genes through assimilation of the preferred carbon source. CCA occurs when the expression of certain operons is stimulated in the presence of preferred carbon sources.

Numerous operons subjected to catabolite control are categorized into two groups as to the involvement of a global transcription regulator, catabolite control protein A (CcpA), in the molecular mechanism underlying their catabolite control. This review deals first with many operons subject to CcpA-dependent catabolite control, and then with ones subject to CcpA-independent catabolite control.

I. CcpA-Dependent Catabolite Control

1. Elucidation of the signal transduction mechanism underlying catabolite control involving CcpA

More than a third of a century ago, a general CCR mechanism based on the disappearance of the stimulatory effect of the cyclic AMP/cyclic AMP receptor protein complex was proposed for *Escherichia coli* and other enteric bacteria.[2] Since many Gram-positive bacteria, including *B. subtilis*, do not possess cyclic AMP, a different general CCR mechanism might operate in these organisms.

Nearly 20 years ago, pioneering work on CCR of α-amylase synthesis in *B. subtilis* resulted in the identification of two constituents of the major catabolite

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control mechanism: a 14-bp *cis*-acting palindromic sequence (TGTAAGCGTTAACA) subsequently called the catabolite-responsive element (*cre*) for *amyE* located in the promoter region of *amyE*,[3,4] and the CcpA protein, a member of the LacI/GalR family of transcriptional regulators.[5] Mutations in either of these constituents resulted in relief from CCR of *amyE* expression.

Besides the *cre* for *amyE*, such *cre* sequences for the *gnt*,[6,7] *xyl*,[8,9] and *hut*[10,11] operons have been identified in the reading frames of *gntR*, *xylA*, and *hutP* respectively, whereas those for the *acu* and *acs* operons are in their divergent promoter regions.[12] In *ccpA* mutants, expression of the *gnt*,[13,14] *xyl*,[15] *hut*,[11] and *acu*[12] operons, and the *acsA* gene,[12] is relieved from CCR. Specific binding of CcpA to the *cre*s of *gnt*[16] and *amyE*[17,18] with and without a corepressor protein, as described below, has been verified by means of DNase I footprinting[16,17] and methylation protection and interference[18] respectively. So far, a total of 50 *cre*s with a 15-bp sequence (one base addition to the 5′-end) have been identified for various genes and operons of *B. subtilis*; they are listed in Table 1.

In contrast to the situation for *E. coli*, CCR in *B. subtilis* occurs only when the repressing sugar can be converted to certain glycolytic intermediates including fructose-1,6-bisphosphate (FBP).[68–70] Mutants unable to produce FBP do not exhibit CCR of *myo*-inositol dehydrogenase, acetoin dehydrogenase, or gluconate kinase, implying the presence of a common regulatory mechanism underlying CCR in *B. subtilis*. The role of FBP is to stimulate phosphorylation of HPr at Ser-46, which is catalyzed by HPr kinase/phosphatase (HPrK/P), as was verified later.[71–74] (HPr is a histidine-containing protein involved in carbohydrate transport *via* the phosphoenolpyruvate:sugar phosphotransferase system.)[75] Thus HPr-mutants (*ptsH1*) are partially or completely relieved from CCR of several catabolic genes.[15,26,59,76,77] Most enzymes that are relieved from CCR in a *ccpA* mutant are also relieved from CCR in a *ptsH1* mutant, implying that P-Ser-HPr and CcpA are involved in the same CCR mechanism and that they possibly interact with each other. This coincides with the fact that CcpA is a protein synthesized constitutively irrespective of the presence or absence of a preferred carbon source,[14] suggesting that CcpA requires a corepressor, which might be P-Ser-HPr, to exert catabolite repression.

Table 1. *B. subtilis* Genes under Control by Catabolite Repression/Activation, the Corresponding *cre* Sequences for Which Have Been Identified

Gene	Rank/100[a]	Function	*cre* sequence (location)[b]	Transcription initiation base[b]	*cre* localization/function[c]	Reference
Group A (TG/CA)						
ackA	A-15	Acetate metabolism	TTGTAAGCGTTATCA(−156/−142)	(−92)	U/A	19–22
acsA	A-6	Acetyl-CoA synthetase	TTGAAAGCGTTACCA(+7/+21)	(−30)	D/R (1)	12, 23
amyE	A-9	α-Amylase	ATGTAAGCGTTAACA(−125/−111)	(−121)	P/R	3
araB	A-53	Arabinose metabolism	ATGAAAACGATTACA(+679/+693)	(−2167)	D/R (1)	24, 25
bglP	A-8	β-glucoside metabolism	ATGAAAGCGTTGACA(−253/−239)	(−212)	P/R	26, 27
cccA	A-23	Cytochrome *c*-550	TTGTAAGCGTATACA(−188/−174)	(−151)	P/R	28
citM	A-36	Mg^{2+}/citrate transporter	ATGTAAGCGGATTCA(−32/−18)	(−71)	P/R	29
dctP (*ydbH*)	A-10	C4-dicarboxylate transporter	ATGAAAACGCTATCA(−64/−50)	(−41, −42)	P/R	25, 30
dra	A-42	Deoxyribonucleoside metabolism	TTGAAACCGCATACA(+34/+48)	(−29, −31)	D/R (1)	25, 31, 32
galT	A-39	Galactose-1-P metabolism	ATGGAAGCGGATACA(+214/+228)	ND	D/R (1)	25
glpF	A-r19, A-70	Glycerol metabolism	TTGACACCGCTTTCA(−181/−167)	ND	p/R (r)	33
malA (*glvA*)	A-30	6-P-α-glucoside metabolism	TTGTAAACGTTATCA(−28/−14)	(−26)	P/R	34
gntR	A-17	Gluconate metabolism	TTGAAAGCGGTACCA(+107/+121)	(−34)	D/R (2)	16, 35
gntR	A-89	Gluconate metabolism	ATGAAAGTGTTTGCA(−81/−67)!!	(−34)	P/R	36
hutP	A-33	Histidine metabolism	TTGAAACCGCTTCCA(+170/+184)	(−32)	D/R (2)	11, 37
ilvB	A-4	Branched-chain amino acid synthesis	ATGAAAGCGTATACA(−578/−564)	(−482)	U/A	38, 39
iolB	A-22	Inositol metabolism	ATGAAAACGTTGTCA(+668/+682)	(−1710)	D/R (2)	40, 41
kdgA	A-41	Hexuronate metabolism	ATGGAAGCGCTGACA(+355/+369)	(−2084)	D/R (1)	25, 42
kduI	A-34	Galacturonate degradation	TTGAAACCGTTACCA(−65/−51)	(−63)	P/R	43
lcfA	A-5	Acyl-CoA synthetase	ATGAAAACGTTATCA(+412/+426)	ND	D/R (1)	25, 44, U[d]
lcfB (*yhfL*)	A-68	Acyl-CoA synthetase	ATGACAACGTTTGCA(+406/+420)	(−54)	D/R (1)	44, U[d]
phoP	A-3	Response regulator of PhoR/PhoP	ATGAAAGCGCTATCA(−161/−147)	$(-175)P_{A6}$	P/R	45
resB	A-Out	Respiration	TTGGTAACGGTTACA(+1152/+1166)	(−566)	D/R (3)(r)	46
treP	A-87	Trehalose metabolism	GTGAAAACGCTTGCA(+317/+331)	(−46)	D/R (2)	25, 47
uxaC (*yjmA*)	A-2	Glucuronate metabolism	ATGAAAGCGTTATCA(+1177/+1191)	ND	D/R (1)	25, 48
xylA	A-43	Xylose metabolism	TTGGAAGCGCAAACA(+35/+49)	ND (not 168)	D/R (2)	9, 25
yobO	A-37	Phage-related function	ATGTAAGCGGATTCA(+1178/+1192)	ND	D/R (2)	25
yxkJ	A-99	Citrate/malate transporter	TTGCAAACGGATACA(+28/42)	ND	D/R (1)	25, 49

Continued

Table 1. *Continued*

Gene	Rank/100[a]	Function	*cre* sequence (location)[b]	Transcription initiation base[b]	*cre* localization/function[c]	Reference
Consensus sequence			WTGNAANCGNWWNCA			
Group B						
abnA	B-3	Arabinose metabolism	TTGTAAGCGCTTTCT(−38/−24)	(−119)	D/R	50
acoA	B-Out	Acetoin metabolism	ATGTAAGCGTTTGCT(+434/+448)	(−41)	D/R (2)	25
acoR	B-2	Activator for *acoABCL*	TTGAAAGCGCTTTAT(−67/−53)	ND	p/R	51
acuA	B-6	Acetoin metabolism	TTGAAAACGCTTTAT(−75/−61)	(−41)	P/R	12, 19
araA	B-15	Arabinose metabolism	TTGAAAGCGTTTTAT(−38/−24)	(−97)	D/R	24, 52
araE	B-50	Arabinose metabolism	ATGAAAACGCTTTAC(−38/−24)	(−102)	D/R	52, 53
ccpC (*ykuM*)	B-Out	Catabolite control protein C	AAGAAAGCGCATACA(−108/−94)	ND (P2)	D/R	54
citS	B-Out	Sensor kinase of CitS/CitT	TTGATAACGCTTTCG(+1298/+1311)	(−28)	D/R (2)	55
citZ	B-Out	Citrate synthase	ATGTAAGCATTTTCT(−114/−100)	(−194)	D/R	56
cydA	B-Out	Cytochrome *bd* oxidase	TTGAAATGAATCGTT(−222/−208)	(−193)	P/R	57
iolA	B-22	Inositol metabolism	TTGAAAGCGTTTAAT(−106/−92)	(−191)	D/R	40, 41
levD	B-67	Levan metabolism	ATGAAAACGCTTAAC(−80/−66)	(−29)	U/R	58, 59
mmgA	B-53	Mother-cell fatty acid degradation	TTGTAAGCGCTGTCT(−37/−23)	(−50)	P/R	60
msmX	B-r11	Sugar ABC transporter	AAGAAAGCGTTTACA(−35/−21)	ND	p/R	25, 49
pta	B-81	Acetate metabolism	ATGAAAGCGCTATAA(−100/−86)	(−37)	U/A	61, 62
resA	B-Out	Thiol-disulfide oxidoreductase	GTAAAAACGCTTTCT(−104/−90)	(−24)	U/R	46
rocG (*yweB*)	B-65	Glutamate dehydrogenase	TTTAAAGCGCTTACA(−50/−36)	(−121)	D/R (r)	63
sigL	B-63	σ^{54} (σ^{H}-dependent)	TGGAAAACGCTTTCA(+564/+578)	ND	D/R (3)	64
xsa	B-45	Arabinose metabolism	TTAAAAGCGCTTACA(−100/−86)	(−101)	P/R	50
xynP (*ynaJ*)	B-10	β-xyloside H^+-symporter	TTGAAAGCGCTTTTA(−99/−85)	(−321)	D/R	65
fadN (*yusL*)	B-19	Fatty acid β-oxidation	ATGAAAGCGCTTATT(+1036/+1050)	(−70)	D/R (1)	44, U[d]
yxjC	B-12	β-Hydroxybutyrate metabolism	TTGTAAACGCTTTCT(−41/−27)	ND	p/R	25, 49
Consensus sequence			WTGAAARCGYTTWNN			

[a]Ranking of *cre* sequences was performed by means of a web-based *cis*-element search of the *B. subtilis* genome (http://dbtbs.hgc.jp/motiflocationsearch.html, N. Sierro and K. Nakai, unpublished)[66,67] using 28 and 22 *cre* sequences with and without a TG/CA set (Groups A and B) as entry sequences respectively. "Out" and "r" denote "out of the 100th rank" and "reverse sequence of *cre* sequence" respectively.

[b]Number(s) indicate the position of the *cre* sequence or the transcription start base relative to the first base of the translation start for the gene. ND in the "transcription initiation base" column denotes "not determined."

[c]"U," "P," and "D" denote the *cre* localization in the upstream, promoter, and downstream regions of the transcription initiation bases respectively. "A" and "R" indicate catabolite "activation" and "repression" respectively. Also, the lower-case "p" and "r" denote the *cre* location in the presumed promoter region and in the reverse *cre* direction as to transcription respectively. Of the *cre* sites located downstream of the transcription initiation bases, these associated with the numbers in parentheses are located in the protein-coding regions of the target genes. When the bases of positions +1, +4, +7, +10, and +13 of the 15-base *cre* sequences correspond to the first, second, and third bases of codons in the protein-coding frames of the target genes, the numbers in parentheses, (1), (2), and (3), are assigned respectively.

[d]"U" indicates "unpublished results by H. Matsuoka and Y. Fujita.

A specific interaction between CcpA and P-Ser-HPr was indeed demonstrated by retarded elution of P-Ser-HPr from CcpA-carrying columns[78] and later by nuclear resonance measurements.[79] In 1995, a complex of CcpA with P-Ser-HPr was verified to recognize the *cre* of the *gnt* operon with high affinity in footprinting experiments.[16] This finding led to the present model for the molecular mechanism underlying CCR in *B. subtilis* (Fig. 1). High affinity binding of the complex of CcpA and P-Ser-HPr to the *amyE cre* has been confirmed by circular dichroism spectroscopy.[79] Moreover, the structure of the CcpA-(P-Ser-HPr)-*cre* complex has been determined.[80] CcpA, HPr with Ser-46, and HPr kinase/phosphatase are well conserved among low-GC Gram-positive bacteria, suggesting that this molecular mechanism underlying CCR is operative in these bacteria.

As shown in Fig. 1, an HPr-like protein (Crh) is another corepressor of CcpA, found during the *B. subtilis* genome sequencing project.[81] While Crh contains conserved Ser-46, which can be phosphorylated with ATP and HPrK/P, it lacks the active site His-15,[82] so Crh is active only in catabolite control. Inactivation of the *crh* alone does not affect CCR or CCA, but the residual CCR observed in *pstH1* mutants disappears when the *crh* is disrupted or a *crh1* mutation (replacement of Ser-46 with Ala) is introduced.[20,23,32,33,41,45,55,58,61,63,65,82,83] The question of how P-Ser-HPr and P-Ser-Crh contribute to CCR and CCA with different efficiencies has not been clearly answered yet, although a Crh-specific function in the regulation of expression during growth on substrates other than carbohydrates was recently revealed,[84] probably because of the drastically higher amount of HPr than of Crh during growth on carbohydrates.[85] Also, P-Ser-Crh displays altered binding to CcpA to effect catabolite control.[86] Although the genomes of many Gram-positive bacteria have been sequenced, Crh has been found to be present only in bacilli, suggesting that Crh-medicated catabolite control with Crh as a corepressor of CcpA is specifically operative in this genus.

2. Characterization of cre *sequences and transcription regulation*

The 50 *cre* sequences that have been experimentally identified to date are listed in Table 1. Extensive base substitution analysis of a *cre* sequence for *amyE* has revealed the consensus sequence of TGWNANCGNTNWCA.[4] Genome-wide analysis of *cre* sequences has led to the proposal of a similar but longer consensus sequence, WWTGNAARCGNWWWCAWW[25] (the underlined sequence corresponds to the *cre* sequence listed in Table 1). This analysis led to the following three proposals: (i) Lower mismatching of *cre* sequences with the consensus sequence is required for *cre* function. (ii) Although *cre* sequences are partially palindromic, lower mismatching in the same direction as that of transcription of the target genes is more critical for *cre* function than in the inverse direction. (iii) However, a more palindromic nature of *cre* sequences is desirable for better functioning. The left-side TG of the two palindromic consensus sequences is likely to be required for pairing with the right-side CA, resulting in proper binding of the complex. In fact, replacement of the last C with another base rendered many *cre*s inoperative, as initially verified for the *cre*s of *amyE*,[4] *gntR*,[16] and *hutP*.[11] However, this pairing is likely compensated for by another pairing between the 5′ and 3′ parts of a *cre* sequence, as demonstrated for the *cre* sequence of *iolA*, TTGAAAGCGTTTAAT;[41] the underlined Ts, which can be paired with the underlined As, are indispensable for the *cre* function. Thus the *cre* sequences listed in Table 1 can be classified into two groups (A and B), *viz.*, ones with and without a TG-CA palindromic pair in their *cre* sequences, giving concensus sequences (WTGNAANCGNWWNCA and WTGAAARCGYTTWNN) respectively (Table 1).

Table 1 also indicates the location of each *cre* from the translation initiation base of the gene closest to it in its target operon together with the transcription initiation base of the operon, if known. Depending on the location of the *cre* sites, the binding of the CcpA/P-Ser-HPr complex (or P-Ser-Crh) to them can regulate transcription in different manners (activation, repression, and roadblock). The protein complex binding to a *cre* located upstream of the promoter results in transcription activation, *viz.*, CCA, as for *ackA*,[20,21] *pta*,[61,62] and *ilvB*.[38] This CCA appears to involve direct interaction of the complex with RNA polymerase, as deduced from the face-of-the-helix dependence of the *cre* sites of *ackA*[20] and *ilvB* (S. Tojo and Y. Fujita, unpublished). It is interesting that CCA of *ackA* involves not only the *cre* but also an approximately 20-bp region immediately upstream of it.[21] CCA of *ilvB*[38] and *pta* appear also to require some region upstream of their *cre*s. The *lev* operon is transcribed from the "−12, −24" promoter recognized by RNA polymerase containing σ^L, the *cre* site of which is located 43 bases upstream of the transcriptional initiation base.[58,59] Expression of the *lev* operon is positively regulated by the transcriptional activator LevR, which binds to an activating sequence upstream of the *cre*.[59] The binding of the protein complex to the *lev cre* presumably prevents activation by the LevR interaction with RNA polymerase through DNA looping. Moreover, a *cre* site of the *res* operon is located more than 70 bases upstream of the transcription initiation base, to which the binding of the protein complex evokes negative regulation of *res* expression,[46] but the molecular mechanism underlying this negative regulation is not known.

The CcpA/P-Ser-HPr (or P-Ser-Crh) complex binds to a *cre* overlapping the promoter, interfering with the binding of the transcription machinery, as for *amyE*,[3] *bglP*,[27] *cccA*,[28] *dctP*,[30] *glpF*,[33] *phoP*,[45] and *acuA*.[19] The binding of the protein complex to a *cre* site located well downstream of the transcription initiation base is considered to block transcription elongation, as for most of the other operons listed in Table 1. This transcription roadblock was first demonstrated for the repression of *E. coli purB* containing an operator interacting with PurR in its reading frame.[87] PurR as well as CcpA belong to the LacI/GalR family of bacterial regulatory proteins, which are supposed potentially to possess the ability to cause this transcription roadblock. CCR of the *gnt* operon carrying a *cre* in the *gntR*-coding region is partially promoter-independent, and the amounts of the transcripts containing regions downstream of the *cre* decrease considerably on the addition of glucose,[7] implying that transcriptional roadblock might be involved in this catabolite repression. This is supported by the finding[88] that a mutation of *mfd* encoding a transcription-repair coupling factor, Mfd,[89] relieves CCR of *hut* and *gnt* expression at the *cis*-acting *cre* sequences located downstream of their transcriptional start sites, but does not affect CCR at the promoter-proximal *cre* sites, such as in *amyE*[3] and *bglP*,[27] suggesting that the Mfd protein displaces RNA polymerase stalled at downstream *cre* sites to which the CcpA/P-Ser-HPr (or -Crh) is bound. Nonetheless, CCR of *acsA* expression is not affected by an *mfd* mutation in spite of the location of the *acsA cre* 44 bp downstream of the *acsA* transcriptional initiation sites, but CCR is relieved by it if the *cre* is placed 161 bp downstream of the initiation site.[23] Hence, transcription roadblock occurring near the transcription initiation base might not require the Mfd protein. Mfd has also been found to be involved in CCR of *dra-nupC-pdp* expression.[32] Furthermore, CCR of *sigL* expression is probably exerted by transcription roadblock through CcpA binding to a *cre* in the *sigL*-coding region.[64] Contrary to these findings, CCR of *xyl* expression through CcpA-binding to a *cre* in the *xylA*-coding frame has been reported to be unlikely to be regulated by a roadblock mechanism.[90]

Among 50 *cre*s experimentally identified, 20 are located in the protein-coding regions of the target genes, so it would be interesting to know where these 20 *cre* sequences are localized in the three possible protein-coding frames. As shown in Table 1, the bases at positions +1, +4, +7, +10, and +13 of WTGNAARCGNWWWCA correspond to the first and second bases of the codons in all cases except for a *cre* in *sigL*. The bases at these positions are W, N, or R, allowing more flexibility of the base species, whereas the first and second bases of the codons in the protein-coding frames of the target genes require less flexibility. The other bases of the *cre* consensus sequence are conserved, frequently corresponding to the third bases of codons in the protein-coding frames, where more base degen-

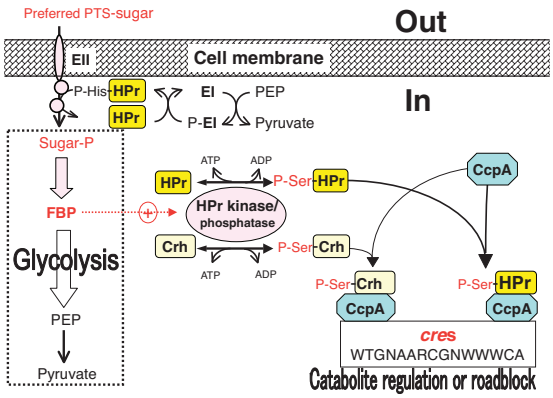

Fig. 1. The Molecular Mechanism of Carbon Catabolite Repression and Activation in *Bacillus subtilis*.

The uptake of a preferred carbohydrate (PTS-sugar), such as glucose, fructose, or mannose, leads to an increase in the FBP concentration in the cell, which triggers ATP-dependent HPr kinase/phosphatase-catalyzed phosphorylation of HPr and Crh at Ser-46. Only the seryl-phosphorylated forms of HPr and Crh are capable of binding to CcpA. The P-Ser-HPr/CcpA and P-Ser-Crh/CcpA complexes can bind to the catabolite responsive elements, *cre*, to cause CCR or CCA, depending on the position of the *cre*. The consensus sequence for the *cre* sequence is WTGNAARCGNWWWCA.[25)] If a *cre* is properly located upstream of the −35 region of the promoter, the complex (P-Ser-HPr/P-Ser-Crh and CcpA) interacting with RNA polymerase causes CCA. The complex binds to a *cre* located in the promoter region, resulting in transcription repression, whereas that bound to *cre* located well downstream of the transcription initiation base evokes transcription roadblock. Similar mechanisms are presumably operative in most other low-GC Gram-positive bacteria, with the proviso that Crh has been found only in bacilli so far.

eracy is allowed. This implies an elegant harmony between the establishment of a *cre* sequence and the evolution of a functional protein encoded by a catabolite-repressive gene.

3. Metabolic networks mediated by CcpA

Determination of the complete genome of *B. subtilis*[81)] has made possible the detection of many genes that are probably subject to CcpA-mediated CCR and CCA by means of transcriptome and proteome analyses[91–97)] as well as an electronical search for the *cre* sequence in the genome sequence.[25,93)] Transcriptome and proteome analyses have revealed that out of the nearly 1,000 of the *B. subtilis* 4,107 protein genes whose expression in cells growing in a nutrient sporulation medium can be detected on DNA microarrays or 2D gels, roughly 10% are repressed or activated more than 3-fold upon the addition of glucose to the medium,[92)] which implies that several hundred genes might be regulated by glucose. More than a few of the candidate glucose-regulated genes examined in the above transcriptome and proteome analyses were experimentally proven to be under CcpA-mediated CCR or CCA through identification of their *cre* sequences. In total, 50 *cre* sequences including those described above, have been experimentally identified so far (Table 1). The *cre* sequences belonging to groups A and B have been separately subjected to a web-based *cis*-element search on the *B. subtilis* genome (http://dbtbs.hgc.jp/motiflocationsearch.html, N. Sierro and K. Nakai,

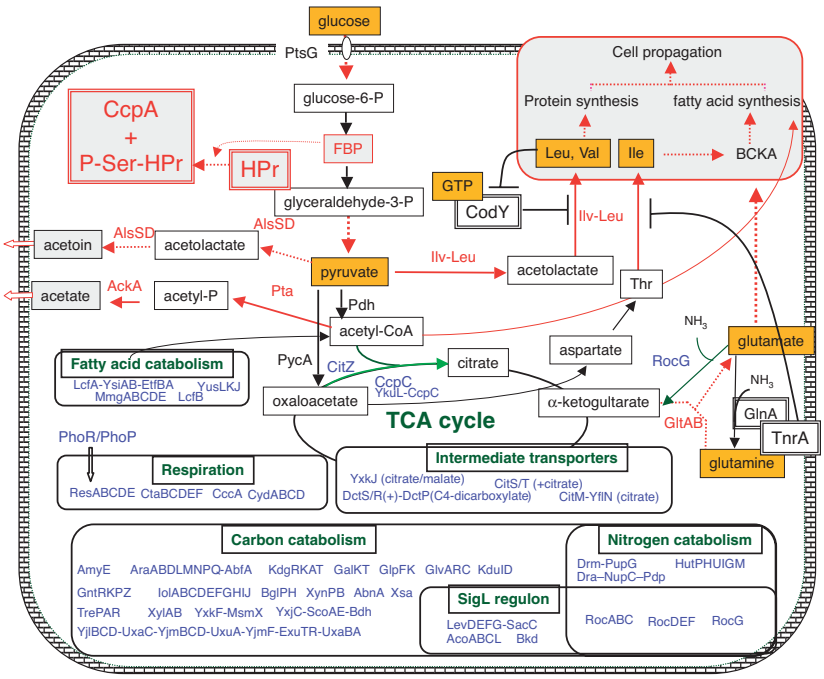

Fig. 2. CcpA-Mediated Metabolic Networks in *Bacillus subtilis*.

The genes and operons subject to CCA mediated by the complex of CcpA and P-Ser-HPr are indicated in red. This complex activates the *ackA*[20)] and *pta*[61)] genes directly, and the *alsSD* operon[99,100)] indirectly, which are involved in the formation of acetate and acetoin, the major extracellular products of *B. subtilis* grown on glucose respectively. It also triggers expression of the *ilv-leu* operon, which is involved in the biosynthesis of branched-chain amino acids, directly.[38,39)] Besides, the complex indirectly triggers the expression of the *gltAB* operon encoding glutamate synthase.[101)] The pathways that are under CCA directly and indirectly mediated by CcpA are indicated by straight and dotted red arrows respectively. On the other hand, the complex of CcpA and P-Ser-HPr represses numerous genes and operons, indicated in blue, which include many genes invoved in carbon, nitrogen, and phosphate metabolism, *citZ* coding for the entrance enzyme of the TCA cycle, several genes encoding TCA cycle intermediate transporters, and the genes involved in respiration. Thus the CcpA-mediated metablic networks play a major role in the coordinated regulation of catabolism and anabolism to ensure optimum cell propagation under given growth conditions. The details are given in the text.

unpublished),[66,67] each search providing the rank numbers of the respective *cre*s out of the 100 *cre* sequences searched for. This ranking is indicated in Table 1, and it shows that seven *cre*s are out of rank. Out of the more than 150 candidate *cre*s resulting from the above two *cre* searches, at least 100 are supposed to function, because of the high sequence-dependency of this *cis*-element (*cre*), as described above. Therefore, this microorganism is assumed to carry nearly 150 *cre*s to regulate roughly 300 genes assuming that two genes are under the control of each *cre* on average. The results of the transcriptome and proteome analyses described above imply various connections of central control of carbon metabolism with various metabolic networks, but the sections below deal only with networks connecting the control of carbon metabolism with other metabolic regulation that has been experimentally verified. Also, it is notable that a CcpA-defective mutant of *B. subtilis* grows at a slower rate in minimal medium with glucose and ammonium as carbon and nitrogen sources than wild-type cells,[98] which indicates an intimate connection between catabolism and anabolism, as described below. The resulting metabolic networks mediated by CcpA are illustrated in Fig. 2.

(i) Carbon catabolite repression (CCR) of the catabolism of secondary carbon and nitrogen sources, and of the anabolism of secondary products

CCR occurs when genes and operons involved not only in catabolism but also in some anabolic processes such as the synthesis of certain extracellular enzymes and secondary metabolites such as antibiotics are not expressed as long as preferred sources of carbon and energy are present. As described above, hundreds of catabolic and anabolic genes and operons are thought to be subject to CCR. Out of the genes and operons involved in carbon and nitrogen metabolism, only those known to be direct targets of the complex of CcpA and P-Ser-HPr are mentioned here (Table 1 and Fig. 2).

The operons involved in the catabolism of secondary carbon sources are as follows (the carbon sources in parentheses): *gntRKPZ* (gluconate),[16,35,36] *xylAB* (xylose),[9] *iolABCDEFGHIJ* (*myo*-inositol),[40,41,102] *trePAR* (trehalose),[25,47,103] *galKT* (galactose),[25] *glpFK* (glycerol),[33] *glvARC* (6-P-α-glucoside),[34] *bglPH* (β-glucoside),[26,27] *yjlBCD-uxaC-yjmBCD-uxuA-yjmF-exuTR-uxaBA* (hexuronate),[25,48] *xynPB* (β-xyloside),[65] *yxjC-scoAE-bdh* (β-hydroxybutyrate),[25,49] *ara-ABDLMNPQ-abfA* (arabinose)[24,52,53] and *abnA xsa* (arabinose),[50] *kdgRKAT* (hexuronate),[25,42] and *kduID* (galacturonate).[43] The *yxkF-msmX* operon probably involved in the transport of unknown sugars has been found to be under CcpA-mediated CCR.[25,49] Besides, the *amyE* gene encoding the extracellular α-amylase hydrolyzing starch,[3,5] and the *levDEFG-sacC* operon encoding a fructose-specific phosphotransferase system and the extracellular levanase hydrolyzing fructose polymers and sucrose,[59,104] are also known to be subject to CcpA-mediated CCR. In addition, some members (*lcfA-fadR-fadB-etfAB*, *fadNAE*, and *lcfB*) of the FadR (formerly YsiA) regulon involved in fatty acid degradation[44,105] are under CcpA-dependent CCR (H. Matsuoka, and Y. Fujita, unpublished observation).

Various amino acids and nucleotides are utilized as carbon and nitrogen sources. The *hutPHUIGM* operon involved in histidine utilization is a direct target of CcpA.[10,11] The *dra-nupC-pdp*[32] and *drm-pupG*[106] operons involved in deoxyribonucleoside metabolism are subject to CcpA-mediated CCR, and the former has been found to be a direct target of CcpA. Moreover, the *sigL* gene encoding a σ^{54}-type factor of *B. subtilis* σ^{L} involved in nitrogen metabolism[104] is a direct target of CcpA.[64] The σ^{L} regulon contains the levanase operon, *levDEFG-sacC*, involved in fructose and levan metabolism,[59,104] three *rocABC*, *rocDEF*, and *rocG* operons associated with arginine catabolism,[107–109] an *acoABCL* operon encoding the acetoin dehydrogenase complex,[51,110] and the seven-cistronic *bkd* operons[111] encoding enzymes involved in leucine and valine degradation. Of these, *acoABCL*, *levDEFG-sacC*, and *rocG* have been experimentally proven to carry the respective *cre* sequences.[25,58,59,63]

(ii) Enhancement of pyruvate assimilation and shutdown of the TCA cycle and respiration

As shown in Table 1, all the *cre*s, except for the three for *ackA*,[20] *pta*,[61] and *ilv*-leu,[38,39] have been found to be involved in negative regulation of catabolic and anabolic genes. The *pta* and *ackA* genes encode phosphotransferase and acetate kinase respectively, which catalyze the conversion of acetyl-CoA to acetate *via* an acetyl–P intermediate. Acetate is one of the major by-products during the growth of *B. subtilis* cells in a rich medium containing rapidly metabolizable carbohydrates such as glucose. The *ilv-leu* operon is one of the major anabolic operons involved in the biosynthesis of branched-chain amino acids (isoleucine, valine, and leucine) (BCAA). In addition, the *alsSD* operon,[99] which is involved in acetoin biosynthesis, is known to be under CcpA-dependent positive regulation,[99,100] but no *cre*-like sequence was found in the promoter region of the *alsSD* operon in our *cre* search involving a web-based *cis*-element search (http://dbtbs.hgc.jp/motiflocationsearch.html),[66,67] implying that an unknown factor might be involved in this positive regulation.[100] The fate of pyruvate is of major importance to the cell.[112] The CcpA-dependent CCA of the *ackA* and *pta* genes and the *alsSD* and *ilv-leu* operons appeared to play similar roles in the reduction of the intracellular concentration of pyruvate accumulated during growth in a rich medium containing rapidly metabolizable carbon sources by means of enhancement of the excretion pathways for acetate (*ackA* and *pta*) and acetoin (*alsSD*), and BCAA biosynthesis from pyruvate (*ilv-leu*).

On the other hand, CcpA represses the expression of *citZ* encoding citrate synthase to condense acetyl-CoA with oxaloacetate directly, and decreases it indirectly through the relief from citrate inhibition of CcpC which is able to repress *citZ* expression.[54,56] This negative regulation of entrance to the TCA cycle allows cells to avoid the production of excess ATP as long as they can obtain enough ATP through glycolysis. Accordingly, the transport of the intermediates of the TCA cycle is also shut down. The *citM-yflN* operon involved in citrate transport is a direct target of CcpA-mediated CCR,[29] which is positively regulated by a two-component regulatory system, CitS/CitT, whose synthesis is also directly repressed by CcpA.[55] The *yxkJ* gene, probably encoding a citrate/malate transporter, is subject to CcpA-

mediated CCR.[25,49] Transport systems for C4-dicarboxylates, such as malate, fumarate, and succinate, are encoded by *dctP*, whose expression is positively regulated by a two-component regulatory system encoded by *dctS*/*dctR* and is subject to CcpA-mediated CCR.[30]

The *B. subtilis* respiration system is severely repressed by glucose. The *resABCDE* operon, indispensable for respiration, encodes a three-protein complex involved in cytochrome *c* biogenesis[113] as well as the ResE sensor kinase and the ResD response regulator, which control electron transfer and other functions in response to oxygen availability.[114,115] This operon is subject to CCR, and is a direct target of CcpA[46] Besides, the *cccA* gene encoding small cytochrome c_{550} has been found to be glucose-repressed through direct interaction with the CcpA/P-Ser-HPr complex.[28] Moreover, the *cydABCD* operon, encoding cytochrome *bd* oxidase, was found to be directly repressed by CcpA.[57]

Transcription of the *resABCDE* operon requires the PhoP/PhoR two-component system.[116] The PhoP-P response regulator directly binds to the *cis*-element of the *res* promoter and is essential for transcriptional activation of the *resABCDE* operon, as well as being involved in repression of the internal *resDE* promoter during phosphate-limited growth. CcpA plays a significant role in transcriptional regulation of the *phoPR* promoter, which is achieved through its direct binding to the *cre* sequence present in *phoPR* promoter A6.[45]

(iii) Major link between carbon and nitrogen regulation

B. subtilis assimilates ammonium through the concerted actions of glutamine and glutamate synthasis. Expression of the *gltAB* operon encoding the latter enzyme depends on the accumulation of glycolytic intermediates, which cannot occur in the *ccpA* mutant,[101] although no candidate *cre* was found in a web-based *cis*-element search (http://dbtbs.hgc.jp/motiflocationsearch.html). Lack of *gltAB* induction is a bottleneck that prevents growth of a *ccpA* mutant on glucose/ammonium media. On the other hand, the *rocG* gene, encoding catabolic glutamate dehydrogenase, has been found to be subject to direct CcpA-dependent glucose repression.[63] The glutamate pool is low in *ccpA* mutants due to a loss of CCR of *rocG*, which contributes to a slow growth rate in glucose/glutamate medium.

(iv) CcpA-mediated catabolite activation (CCA) of branched-chain amino acid (BCAA) biosynthesis

BCAAs are the most abundant amino acids in proteins, and they form the hydrophobic cores of the proteins. Moreover, these amino acids are precursors in the biosynthesis of *iso*- and *anteiso*-branched fatty acids, which represent the major fatty acid species of the membrane lipids in *Bacillus* species.[117] The initial step of isoleucine and of valine synthesis is the condensation of 2-oxobutanoate derived from threonine and pyruvate or two pyruvates, leading to the formation of branched-chain keto-acids.[118] Leucine is synthesized from one of the branched-chain keto acids, *viz.*, α-ketoisovalerate. The *B. subtilis ilv-leu* operon comprises seven genes (*ilvB*, *H*, and *C*, and *leuA*, *B*, *C*, and *D*) necessary for the biosynthesis of BCAAs.[119] Besides the probable necessity that the CcpA-dependent CCA of *ilv-leu* in glycolysis proceed continuously for the draining of accumulated pyruvate, it is notable that this positive regulation links carbon metabolism to amino acid anabolism. Recent global gene expression studies on amino acid availability[120] and CodY regulation,[121] as well as on the metabolic links of *ilv-leu* expression to glucose and nitrogen metabolism,[38,39,98,122] indicate that the *ilv-leu* operon is under direct negative transcriptional control through two major global regulators of nitrogen metabolism (CodY and TnrA).

The CodY protein is a GTP-binding repressor of several genes that are normally quiescent when cells are growing in a nutrient-rich medium.[123] A high concentration of GTP activates the CodY repressor, which serves as a gauge of the general energetic capacity of the cells. CodY is also induced through direct interaction with BCAAs to bind to the promoter regions of target genes, including the *ilv-leu* operon for their repression.[124] Thus *in vivo* BCAA concentrations serve as a gauge of nutrient conditions through the activation of CodY. TnrA is known to activate and also to repress nitrogen-regulated genes during nitrogen-limited growth.[125] When nitrogen sources are in excess, the concentrations of intracellular glutamine and other metabolites are thought to become high enough to cause feedback inhibition of glutamine synthase (GlnA). The feedback-inhibited GlnA captures TnrA to form a protein–protein complex, and thereby abolishes the DNA-binding ability of TnrA. By contrast, during nitrogen-limited growth, TnrA is released from the GlnA-TnrA complex and binds to its specific sites on DNA for the regulation of transcription. Thus, TnrA exerts its regulatory function only in cells grown under nitrogen-limited conditions.[125]

It is notable that the CcpA-dependent CCA of *ilv-leu* is associated with the respective counter-negative regulation mediated by CodY and TnrA under both nitrogen-rich and -limited conditions. To achieve the full growth potential of rapidly metabolizable carbohydrates such as glucose, CcpA tends to enhance the expression of *ilv-leu* to make the cell synthesize more BCAAs for rapid cell growth. However, when enough BCAAs are supplied by a nitrogen-rich medium, negative regulation exerted by CodY interacting with these amino acids overwhelms CcpA-dependent positive regulation to prevent excess synthesis for the maintenance of their appropriate concentrations *in vivo*. The repression of *ilv-leu* expression through CodY is overwhelming regardless of whether CcpA-dependent positive regulation occurs. On the other hand, when cells are grown in a nitrogen-limited medium containing glutamate as the sole nitrogen source, TnrA decreases CcpA-dependent CCA to adjust the amounts of BCAAs in response to a poor nitrogen supply. BCAAs are the most abundant amino acids in proteins, and are precursors for the biosynthesis of *iso*- and *anteiso*-branched-chain fatty acids. The intracellular BCAA concentrations appear to serve as a gauge of nutrient conditions, that is, as a pacemaker of the synthesis of proteins and membranes, probably representing cell growth. Thus, global regulators of cellular metabolism (CcpA, CodY, and TnrA), each of which controls the expression of a certain set of numerous catabolic and anabolic genes, participate in transcription regulation of the *ilv-leu* operon, unlike in the regulation of other biosynthetic pathways, in which only their own feedback systems are generally involved.

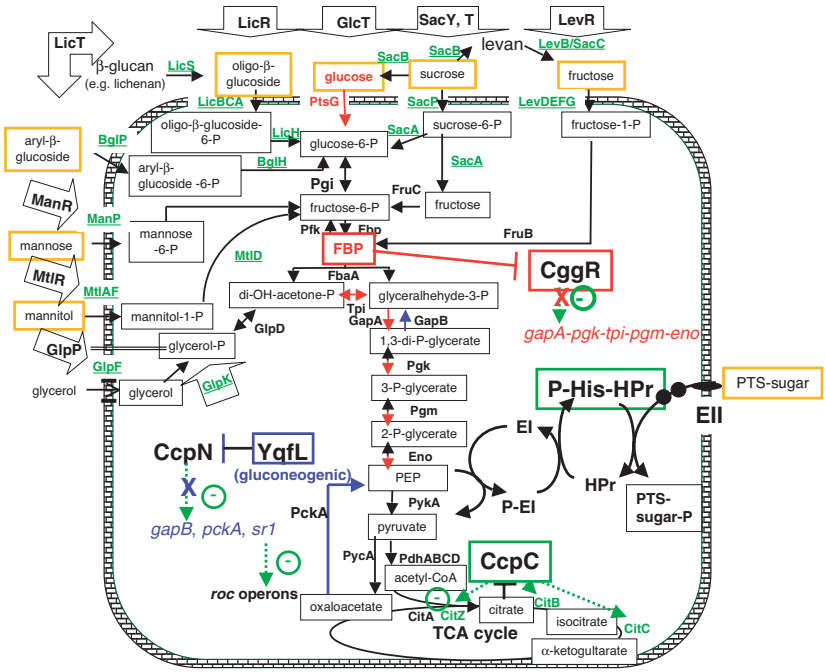

Fig. 3. CcpA-Independent Catabolism Control of Carbon Metabolism in *Bacillus subtilis*.

Enhancement of the glycolytic pathway from dihydroxyacetone-P to PEP in the presence of glucose is achieved by relief of the *gapA-pgk-tpi-pgm-eno* operon from CggR repression (indicated in red). The *in vivo* concentration of fructose-1,6-bisphosphate (FBP) increases upon active transport of glucose through the PTS, which diminishes CggR binding ability as to its *cis*-sequence, CggR being unable to function as a repressor.[126] In contrast, an increase in gluconeogenesis is achieved by relief from CcpN repression of *gapB* and *pckA*, specific to gluconeogenesis, in the interaction of CcpN with active YqfL (indicated in blue).[127] The CcpC protein represses the expression of the *citB* and *citZCH* operons.[128] Its activity is inhibited by citrate.[54,129] Negative regulation through CggR, CcpN, and CcpC is indicated in green. GlcT, SacY, T, LicT, and GlpP are antiterminators for *ptsGHI* encoding EIICBA^{Glc}, HPr, and EI; *sacXY*, *sacPA-ywdA*, and *sacB-levB*; *licS* and *bglPH-yxiE*; and *glpFK* respectively. PTS-sugars, such as glucose, mannose, fructose, sucrose, mannitol, oligo-*β*-glucoside, and aryl-*β*-glucoside, are converted to PTS-sugar-P through the transfer of a phosphoryl group from the phosphorylated forms of EIIs such as PtsG, ManP, LevDEFG, SacP, MtlAF, LicBCA, and BglP respectively. The phosphorylated form of EIIs is produced through phosphoryl transfer from P-His-HPr, which has been formed through successive phosphoryl transfer from PEP and EI. The lower amount of P-His-HPr as to HPr, which is generated through active transfer of the preferred PTS-sugars, such as glucose, results in dephosphorylation of antiterminators of the operons for utilization of less-preferred PTS-sugars (SacT, Y, and LitT) and transcriptional activators (LevR for the *levDEFG-sacC* operon, LicR for *licBCAH*, ManR for *manPA-yjdF*, and MtlR for *mtlAFD*) inhibiting their activity to reduce the expression of the target operon, *viz.*, CCR (indicated in green and underlined). Glycerol kinase (GlpK), which phosphorylates glycerol to glycerol-3-P, is also phosphorylated and activated through phosphoryl transfer from P-His-HPr. The complex of an antiterminator of GlpP with glycerol-3-P binds to the *cis*-sequence to antiterminate *glpFK* transcription. Therefore, the less phosphoylated GlpK, which is produced in the presence of preferred PTS-sugars such as glucose, results in CCR of *glpFK* (indicated in green and underlined). Refer to details and references in the text.

Therefore, BCAA biosynthesis is elaborately regulated according to the cellular energetic and nutritional conditions through the intracellular concentrations of the signal compounds of individual global regulators, such as GTP, BCAAs, glutamine, and fructose-bisphosphate. Thus the elegant regulation of *ilv-leu* most likely plays a central role in linking catabolism to anabolism in the overall metabolism of *B. subtilis*.

II. CcpA-Independent Catabolite Control

Transcriptome and proteome analyses involving *ccpA* mutants[92–95,97] have revealed not only numerous genes whose expression is under CcpA-dependent catabolite control, but also not a few genes under CcpA-independent catabolite control. The individual induction systems of the catabolic genes are most likely involved in their CcpA-independent CCR, as described for *iol*,[92] except for those exerted by the other catabolite control proteins (CcpB, CcpC, and CcpN) as well as CggR which is involved in the regulation of central glycolytic genes. However, only a few of the CcpA-independent CCR affecting the induction systems of these catabolic operons have been intensively investigated. The networks of CcpA-independent CCR/CCA are described below (see Fig. 3).

1. Catabolite control mediated by CcpB, CcpC, CcpN, and CggR

Three catabolite control proteins (CcpB,[130] CcpC,[128] and CcpN[127]) besides the CcpA protein have been reported. CcpB, a paraloguous protein of CcpA, is involved in the CCR of some catabolic operons, such as *gnt* and *xyl*, especially in cells growing on solid media,[130] and in the CCR of *fad* genes participating in fatty acid degradation (unpublished observation, H. Matsuoka and Y. Fujita), although its role in CCR remains to be investigated. The CcpC protein, a LysR family member, represses the *citB* and *citZCH* operons encoding the first three steps of the TCA cycle (*citZ*, *citB*, and *citC*).[128] *ccpC* is negatively autoregulated, and CcpC activity is inhibited by citrate.[54,129] As described above, CcpA controls the expression of the TCA cycle genes directly by repressing transcription of *citZ* and indirectly by regulating the availability of citrate.[56] Also, CcpA directly represses *ccpC* expression.[54]

The CcpN protein represses *pckA* and *gapB* in the same manner during glycolysis, and these genes encode

specific enzymes for gluconeogenesis, PEP carboxykinase and NADPH-dependent glyceraldehyde-3-P dehydrogenase.[127] *B. subtilis* possesses NADH- and NADPH-dependent glyceraldehyde-3-P dehydrogenases, which are specific for glycolysis and gluconeogenesis and are encoded by *gapA* and *gapB* respectively.[131] This protein also represses *sr1*,[132] which encodes a small non-coding regulatory RNA that inhibits the translation of *ahrC* encoding a transcriptional regulator that activates the *rocABC* and *rocDEF* operons for arginine catabolism and represses the gene cluster for arginine biosynthesis.[108,133–135] CcpN is active when cells are growing on a glycolytic substrate, even if the medium also contains a gluconeogenic substrate.[127] CcpN is inhibited on interaction with YqfL, which is active during gluconeogenesis, and *pckA* and *gapB* as well as *sr1* are derepressed. In contrast, the CggR protein represses the genes encoding the conversion of the three-carbon intermediates of glycolysis (*gapA*, *pgk*, *tpi*, *pgm*, and *eno*), which form the *gapA* operon (*cggR-gapA-pgk-tpi-pgm-eno*). Transcription of the entire *gapA* operon from the *gapA* promoter is repressed by CggR, resulting in the *gapA-pgk-tpi-pgm-eno* transcript due to endonucleolytic cleavage between *cggR* and *gapA*, although the *pgk-tpi-pgm-eno* transcript is constitutively formed from another promoter in the intergenic region between *gapA* and *pgk*.[126,136] FBP is an inhibitor of CggR activity.[126] FBP, in the millimolar range, reduces CggR binding to its *cis*-sequence which is located downstream of the *gapA* promoter.[137] Hence FBP is a very suitable signal for the regulation of the genes for glycolysis, since its concentration is much higher during the utilization of glycolytic carbon sources (more than 10 mM) than under gluconeogenic conditions.[138,139]

The FBP concentration is thus the signal that modulates expression of the enzymes for the conversion of the three-carbon intermediates of glycolysis. It is also the main signal for the CcpA-dependent CCR of numerous genes in the utilization of secondary carbon sources and for the CcpA-dependent catabolite control of several genes of the TCA cycle and the overflow pathways (Fig. 2), as described above. FBP is thus a key signaling molecule in the regulation of carbon metabolism in *B. subtilis*.

2. Catabolite repression (CCR) involving P-His-HPr

P-His-HPr phosphorylates the various EIIAs of the PTS, and is also capable of phosphorylating a catabolic enzyme, glycerol kinase, RNA-binding antiterminators for PTS-sugar utilization operons, and DNA-binding transcription activators in some PTS-sugar utilization systems, all of which contain a specific domain called a PTS regulation domain (PRD). These control mechanisms resulting in CCR are found in bacilli.

(i) Glycerol kinase (GlpK)

The *glpFK* operon, encoding a facilitator of glycerol diffusion (GlpF) and glycerol kinase (GlpK), is induced by binding of the complex of GlpP and glycerol-3-P to the 5′-leader region of the *glpFG* mRNA to prevent the formation of the terminator. Glycerol kinase is activated by phosphoryl transfer from P-His-HPr, the concentration of which decreases during active phosphorylation of glucose.[33] Thus this operon is subjected not only to CcpA-dependent CCR, as mentioned above, but also to this CcpA-independent CCR.

(ii) BglG/SacY-type of antiterminators

B. subtilis contains four homologous antiterminator proteins of the BglG/SacY family. These proteins (SacY, SacT, LicT, and GlcT) positively control the genes involved in the metabolism of carbohydrates that are taken up by the PTS; SacY and T are involved in sucrose metabolism, LicT in the metabolism of oligo-β-glucoside and aryl-β-glucoside, and GlcT in glucose assimilation. SacY and T[140–142] are antiterminators of the *sacB* (encoding levansucrase)-*levB* (endolevanase)-*yveA*, *sacX* ($IIBC^{SacX}$)-*sacY*, and *sacP* ($IIBC^{SacP}$)-*sacA* (sucrose-6-P hydrolase)-*ywdA* operons, whereas LicT[27,143] is an antiterminator of the *licS* gene (endo-1,3-1,4-β-glucanase) and the *bglP* ($IIBCA^{Bgl}$)-*bglH* (6-P-β-glucosidase)-*yxiE* operon, and GlcT[144,145] is that of the *ptsG* ($IICBA^{Glc}$)-*ptsH* (HPr)-*ptsI* (EI) operon. In the presence of the cognate PTS-sugars that activate these antiterminators, they bind to a conserved motif called the ribonucleic antiterminator (RAT), which is present in the untranslated leader-mRNAs of their target genes. This prevents the formation of an overlapping transcriptional terminator, which otherwise abolishes transcription elongation and thus expression of the genes. Each antiterminator controls the expression of a cognate EII of the PTS, which in turn negatively regulates its antiterminator. This involves reversible phosphorylation of the antiterminator by abundant P-EII in the absence of its substrate. In addition, SacT, Y and LicT are also positively controlled through phosphorylation by P-His-HPr of the PTS at a distinct site to be active. This constitutes a CCR mechanism that down-regulates the activities of these antiteminators, because P-His-HPr is efficiently used in the phosphorylation of other preferred PTS-carbohydrates, such as glucose, when they become additionally available.

(iii) LevR and DeoR-type activators with PRDs

B. subtilis possesses four transcription activators containing a PRD (LevR, LicR, ManR, and MtlR). LevR positively controls the expression of the *levDEFG* (encoding $EIIA^{lev}$, $EIIB^{lev}$, $EIIC^{lev}$, and $EIID^{lev}$ respectively)-*sacC* (levanase) operon, which is located just downstream of *levR*. Mutation studies have revealed that inactivation of the general PTS proteins of LevD ($EIIA^{lev}$) and LevE (IIB^{lev}) affect LevR activity. Mutants with inactive $EIIA^{lev}$ or $EIIB^{lev}$ exhibited strong constitutive expression from the *lev* promoter.[146,147] Elevated LevR activity was also observed when the *ptsH* or *ptsI* gene was disrupted, although the effect was significantly lower than with the *lev* mutants.[59] In addition, deletion of *pstHI* in a *levD* or *levE* background diminished expression from the *lev* promoter.[148] From these results it was concluded that similarly to PRD-containing antiterminators, LevR also possesses positive and negative sites of regulation, which are expected to be the targets of phosphorylation by P-His-HPr and $P\text{-}EIIB^{lev}$ respectively. If glucose or other preferred PTS-sugars are present additionally, the phosphoryl group of P-His-HPr is preferentially used in sugar phosphorylation. This mechanism thus leads to CcpA-independent CCR.

PRD-containing DeoR-type regulators (LicR, ManR, and MtlR) are controlled similarly to LevR. LicR, the

positive regulator of the *licBCAH* operon, which encodes a cellobiose-specific PTS and a 6-P-β-glucosidase, binds to a binding site with dyad symmetry preceding the *licBCAH* promoter.[149] Genetic experiments have revealed that LicR is subject to dual control by the PTS. Inactivation of *pstI* and *ptsH* completely prevented LicR activity, whereas mutants devoid of LicA ($EIIA^{Cel}$) or LicB ($EIIB^{Cel}$) exhibited constitutive expression from the *lic* promoter.[150] *B. subtilis* MtlR is the regulator of the *mtlAFD* operon encoding the PTS for mannitol,[151] and it specifically interacts with DNA regions containing the *mtl* promoter.[152] Disruption of the *mtlR* gene prevented the utilization of mannitol, indicating that MtlR functions as a transcription activator of the *mtlAFD* operon. However, the role of PTS-mediated regulation has not been studied yet. The regulation of ManR of *B. subtilis* has not been studied to date, either biochemically or genetically.

III. Concluding Remarks

The HPr protein is the energy-coupling protein of the PTS, which catalyzes the transport of carbohydrates in bacteria. The phosphoryl group of PEP is transferred to EI to form EI-P, and this phosphate in EI-P is then transferred to HPr to produce P-His HPr. Finally, the phosphate is utilized to phosphorylate the PTS-sugar to cross the cell membrane *via* EII (Fig. 1). When *B. subtilis* cells grow on a rapidly-metabolizable carbon source, *e.g.*, a preferred PTS-sugar such as glucose, the intracellular concentration of FBP increases, and this is the signal compound that enhances the indispensable stage of glycolysis from dihydroxyaceton-P to PEP, as follows: The enzymes required for this stage of glycolysis are encoded in the *cggR-gapA-pgk-tpi-pgm-eno* operon. The first gene product of CggR is the repressor of this operon, which is antagonized by FBP which increases under glycolytic growth conditions (Fig. 3). Thus the expression of this operon is derepressed to produce more enzymes essential for glycolysis to proceed efficiently. On the other hand, the increased intracellular concentration of FBP in cells growing on a preferred PTS-sugar is the main signal for CcpA-dependent CCR. As shown in Fig. 1, an increase in the FBP concentration triggers the ATP-dependent HPr kinase/phosphatase-catalyzed phosphorylation of HPr (as well as Crh) at Ser-46 to P-Ser-HPr. P-Ser-HPr is capable of binding to CcpA, and the P-Ser-HPr/CcpA complex can bind to the catabolite responsive element (*cre*) to exert CCR or CCA, depending on its position as to the transcriptional promoter; a consensus sequence for the *cre* sequence is WTGNAARCGNWWWCA.[25] If a *cre* is properly located upstream of the -35 region of the promoter, the P-Ser-HPr/CcpA complex interacting with RNA polymerase causes CCA. The complex binds to a *cre* located in the promoter region, resulting in transcription repression, whereas that bound to a *cre* located well downstream of the transcription initiation base evokes transcription roadblock. The target operons of this CcpA-dependent catabolite control are roughly estimated to number 300. The complex of CcpA and P-Ser-HPr triggers the expression of several genes involved in the formation of acetate and acetoin, which are major extracellular products of *B. subtilis* grown on glucose (Fig. 2). It also triggers the expression of an anabolic operon (*ilv-leu*) involved in the biosynthesis of branched-chain amino acids, which subsequently leads to cell propagation. This complex also represses many genes and operons, which include an entrance gene for the TCA cycle (*citZ*), several transporter genes for TCA cycle-intermediates, some respiration genes, and many catabolic and anabolic genes involved in carbon, nitrogen, and phosphate metabolism, as well as those for certain extracellular enzymes and secondary metabolites. Consequently, it is rationally concluded that FBP is a key signaling molecule in carbon catabolite control of the metabolic network in *B. subtilis*.

P-His-HPr also regulates the gene expression and activities of enzymes involved in the utilization of less-preferred carbon sources (Fig. 3). Glycerol kinase (GlpK) requires phosphorylation by P-His-HPr to be fully active. Glycerol-3-P, the product of the kinase, is required for antitermination of the glycerol utilization operon (*glpFK*). The preferred PTS-sugars, such as glucose, prevent this phosphorylation, leading to a switch from glycerol- to PTS-sugar utilization. Moreover, P-His-HPr phosphorylates and thereby enhances the activity of transcriptional regulatory proteins, which function as antiterminators at the RNA level (SacT, Y, and LicT) or as transcriptional activators binding to DNA, such as LevR, which enhance expression of the utilization operons of less-preferred PTS-sugars, such as the degradation products of levan and lichenan. This mechanism thus down-regulates the activity of such regulators due to the decreased level of P-His-HPr through active phosphorylation of the preferred PTS-sugars, such as glucose, transporting them into the cells, when these PTS-sugars become available in addition to the cognate substrate. Thus this mechanism leads to CcpA-independent CCR. Moreover, when cells grow on gluconeogenic carbon sources, another catabolite control protein (CcpN), a repressor for gluconegenesis-specific *gapB* and *pckA* encoding NADPH-dependent glyceraldehyde-3-P dehydrogenase and PEP carboxykinase, is antagonized through interaction with YqfL, which is active only during gluconeogenesis. Thus *gapB* and *pckA* are relieved from CcpN repression when the cells shift from glycolytic to gluconeogenic growth, and their expression is under CcpA-independent CCR.

I have reviewed carbon catabolite control of the metabolic network in *B. subtilis.* Further information on this topic can be obtained from other recent reviews.[153–158]

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