Supporting Information

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SI Materials and Methods

Growth Conditions, Strains, and Plasmids. Bacteria were routinely grown in LB containing antibiotics (50 or 100 μg/mL ampicillin, 10 μg/mL tetracycline, 20 μg/mL chloramphenicol, 40 μg/mL kanamycin) when necessary. For determination of β-galactosidase activities, *Escherichia coli* strains were grown in M9 minimal medium containing 1% (wt/vol) glycerol, proline (20 μg/mL), thiamine (1 μg/mL), casamino acids [0.66% (wt/vol)] and the appropriate antibiotics. Where indicated, 1 mM isopropyl-β-D-1-thiogalactopyranoside (IPTG) was added as inducer for *tacOP* and 7 mM D-salicin was added as substrate for BglF.

The strains and plasmids used are described in Table S1. Oligonucleotides are listed in Table S2. Plasmid pFDX4732 was constructed by ligating the AseI–HindIII fragment of plasmid pFDX3214 encompassing the *fruB* gene between the AseI/HindIII sites of plasmid pFDX3852. The series of plasmids pFDX4733–pFDX4739 was constructed by ligation of the XhoI–SalI fragment of plasmid pFDX4291 encompassing the pSC101 origin of replication and the *cat* gene to the SalI–SalI fragments encompassing the P_{tac} expression cassettes of the following plasmids, which resulted in the plasmids given in parentheses, respectively: pFDX3161 (pFDX4733), pFDX3851 (pFDX4735), pFDX3852 (pFDX4736), pFDX3221 (pFDX4737), pFDX4732 (pFDX4738), and pFDX3214 (pFDX4739). For construction of plasmids pGP1286 and pGP1319 carrying *strep–bglG* and *strep–*s_{tag}*–bglG* under T7 promoter control, respectively, *bglG* was amplified from chromosomal DNA using primers FR108/FR109 (for *strep–bglG*) and primers FR181/FR109 (for *strep–*s_{tag}*-bglG*) and cloned between the SacI and BamHI sites of plasmid pGP172. Plasmids pGP1309 and pGP1320 carrying *strep–bglG–H208A* and *strep–*s_{tag}*–bglG–H208A* under T7 promoter control, respectively, were constructed in the same manner, but with the difference that plasmid pFDX4221 was used as template for PCR. Plasmid pGP438 carrying *strep–ptsH* (*ptsH* from *Bacillus subtilis*) under T7 promoter control was constructed by amplification of the *ptsH* gene from the *B. subtilis* chromosome using primers SHU54 and SB46 and subsequent cloning of the resulting PCR fragment between the SacI and BamHI sites of plasmid pGP172. For construction of plasmids pGP1287, pGP1288, and pGP1299 carrying His_{10}–*ptsH*, His_{10}–*fruB* and His_{10}–*ptsI* under P_{tac} control, respectively, the respective genes were amplified by PCR using primers FR120/FR121 (*ptsH*), FR122/FR123 (*fruB*), and FR128/FR129 (*ptsI*). Subsequently, the PCR fragments were used to substitute the NheI–XbaI fragment encompassing *ptsN* in plasmid pBGG190.

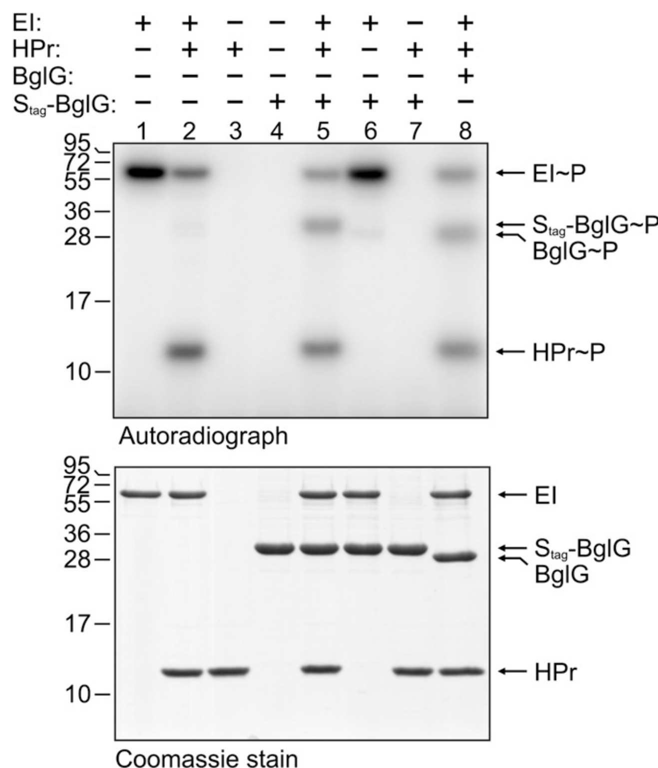
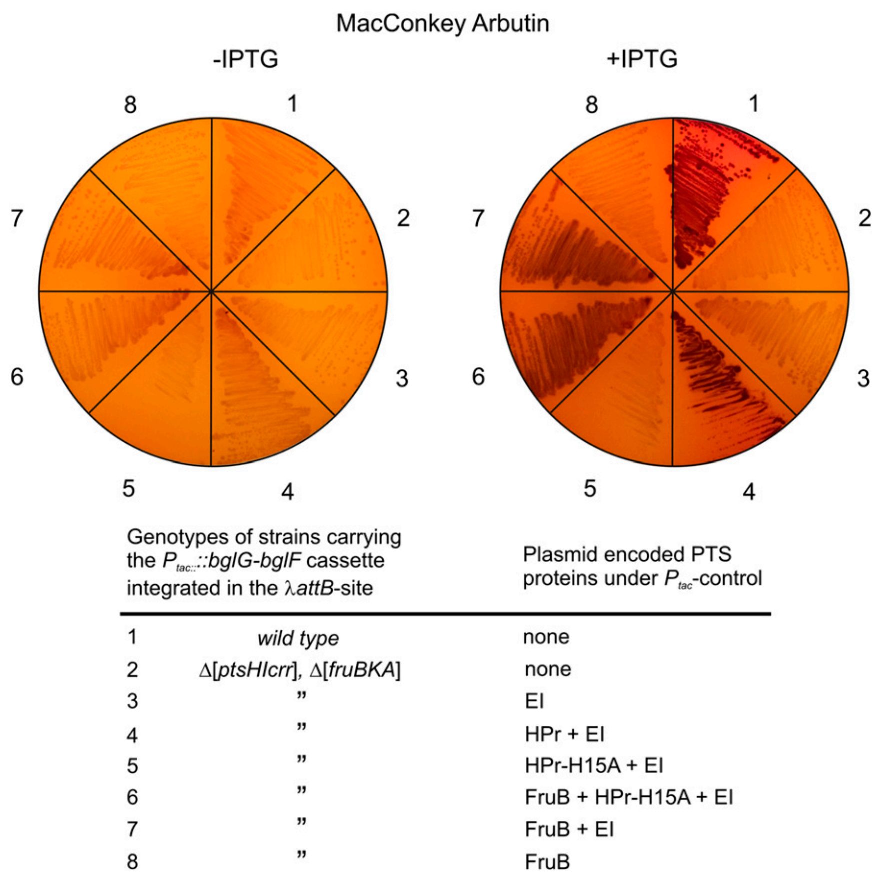


Fig. S1. PEP and EI-dependent phosphorylation of the Strep–S_{tag}–BglG fusion protein by HPr in vitro. Phosphorylation assays were carried out using either [^{32}P]-PEP (*Upper*) or 1 μM cold PEP (*Lower*). In these assays, purified His_{10}–EI, His_{10}–HPr, and Strep–S_{tag}–BglG were present as indicated. In the last lane, Strep–BglG was used as a control to demonstrate the increased molecular weight caused by the additional S_{tag} in Strep–S_{tag}–BglG. After, the assay proteins were separated on 15% SDS-polyacrylamide gels and subsequently analyzed by phosphoimaging (*Upper*) or staining with Coomassie brilliant blue (*Lower*). Positions of the molecular weight marker are given at *Left*.



Genotypes of strains carrying the P_{tac}::*bglG-bglF* cassette integrated in the *λattB*-site		Plasmid encoded PTS proteins under P_{tac}-control
1	*wild type*	none
2	Δ[*ptsHIcrr*], Δ[*fruBKA*]	none
3	"	EI
4	"	HPr + EI
5	"	HPr-H15A + EI
6	"	FruB + HPr-H15A + EI
7	"	FruB + EI
8	"	FruB

Fig. S2. FruB can substitute for HPr in aryl-β-glucoside transport. Strains R1752 (1, wild type) and R1977 (2–8; Δ[*ptsH, ptsI, crr*]::*neo*, Δ[*fruB, fruK, fruA*]) were transformed with low copy plasmids carrying P_{tac}-controlled genes coding for the PTS proteins as indicated. These plasmids were pFDX283 (sectors 1 and 2), pFDX3161 (sector 3), pFDX3851 (sector 4), pFDX3852 (sector 5), pFDX4732 (sector 6), pFDX3221 (sector 7), and pFDX3214 (sector 8). Strains also carried an artificial P_{tac}::*bglG-bglF* operon integrated into the chromosomal *λattB* site, whereas the natural *bgl* operon was deleted. To allow for regulated expression of the genes under P_{tac} control, the strains additionally carried the compatible plasmid pFDX3158 as source for LacI repressor. The various transformants were streaked on MacConkey indicator plates containing the aryl-β-glucoside arbutin and IPTG for induction of P_{tac}-controlled genes as indicated (−IPTG, *Left*; +IPTG, *Right*). A dark red color of the colonies indicates uptake and utilization of arbutin. It should be noted that these strains can transport the aryl-β-glucoside salicin, but are unable to catabolize this sugar due to the absence of the phospho-β-glucosidase BglB, which is encoded by the third gene of the *bgl* operon. Arbutin, which is also transported by BglF, is catabolized by the phospho-β-glucosidase BglA, encoded elsewhere, which is constitutively expressed (1). BglA is able to degrade phosphoarbutin, but not phosphosalicin (2). The data show that presence of EI and HPr (sector 4) or EI and FruB (sectors 6 and 7) allows transport and utilization of arbutin (*Right*).

1. Junne T, Schnetz K, Rak B (1990) Location of the *bglA* gene on the physical map of *Escherichia coli*. *J Bacteriol* 172:6615–6616.
2. Prasad I, Young B, Schaefler S (1973) Genetic determination of the constitutive biosynthesis of phospho-β-glucosidase A in Escherichia coli K-12. *J Bacteriol* 114:909–915.

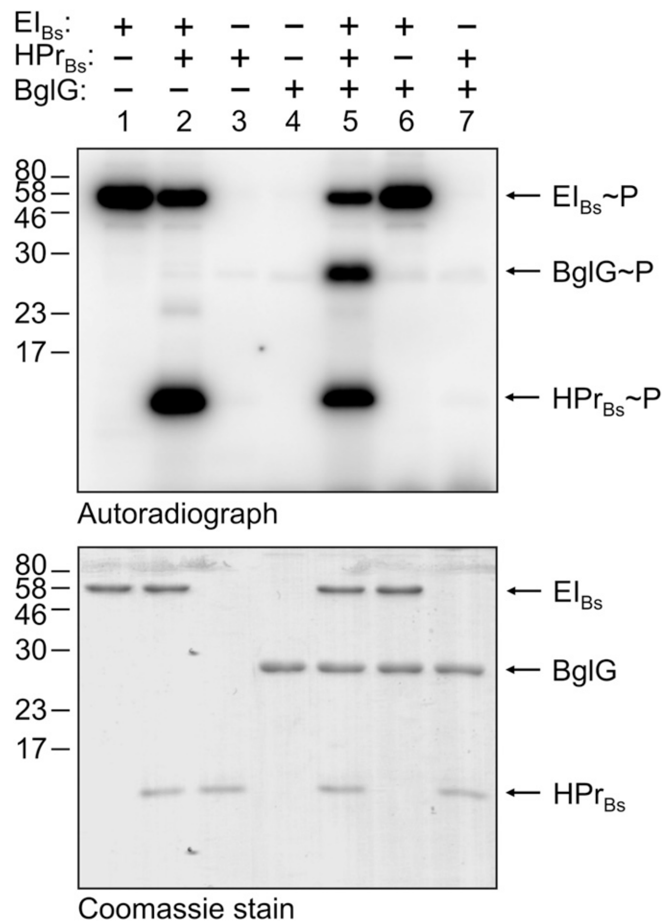


Fig. S3. BglG also becomes phosphorylated in vitro by HPr from *Bacillus subtilis*. It has previously been shown that BglG also gains activity, when EI and HPr in *E. coli* are replaced by their homologs from *B. subtilis*. This activation of BglG occurred in a strain lacking the *fruBKA* operon and required the His15 phosphorylation site in HPr, suggesting that HPr from *B. subtilis* is able to activate BglG by phosphorylation like its *E. coli* homolog (1). To test whether phosphorylgroup transfer is indeed possible between these two proteins, we carried out PEP-dependent in vitro phosphorylation assays using purified recombinant His_6–EI and Strep–HPr, both from *B. subtilis* (EI_{Bs} and HPr_{Bs}). Proteins were tested in various combinations as indicated. Assays were carried out using either [^{32}P]-PEP (*Upper*) or 1 μM cold PEP (*Lower*). Proteins were subsequently separated by denaturing gel electrophoresis using 15% SDS-polyacrylamide gels. Gels were analyzed by phosphoimaging (*Upper*) or staining with Coomassie brilliant blue (*Lower*). Positions of the molecular weight marker are given at *Left*. The data show that both EI_{Bs} and HPr_{Bs} became phosphorylated when incubated together with [^{32}P]-PEP (lane 2). When Strep–BglG was additionally present, it also became phosphorylated, but not when it was incubated with EI_{Bs} or HPr_{Bs} alone (lanes 5–7). In conclusion, BglG can also be phosphorylated by HPr from *B. subtilis*.

1. Reichenbach B, Breustedt DA, Stülke J, Rak B, Görke B (2007) Genetic dissection of specificity determinants in the interaction of HPr with enzymes II of the bacterial phosphoenolpyruvate:sugar phosphotransferase system in *Escherichia coli*. *J Bacteriol* 189:4603–4613.

Table S1. ***E. coli*** **strains and plasmids used in this study**

Name	Genotype	Reference
Strains		
FT1/pLysS	BL21(DE3) Δ[*ptsH, ptsI, crr*]::*neo*/pLysS, *cat*	(1)
R1279	CSH50 Δ(*pho-bgl*)201 Δ(*lac-pro*) *ara thi*	(2)
R1752	as R1279, but *attB::tacOP-bglG-bglF* (*bla*)	(3)
R1969	as R1279, but Δ[*ptsH, ptsI, crr*]::*neo*, Δ[*fruB, fruK, fruA*]	(4)
R1977	as R1279, but *attB::tacOP-bglG-bglF* (*bla*), Δ[*ptsH, ptsI, crr*]::*neo*, Δ[*fruB, fruK, fruA*]	(3)
R2013	as R1279, but *attB::tacOP-bglG-bglF* (*bla*), Δ[*ptsH, ptsI, crr*]::*neo*	(3)
Plasmids		
pAG3	*His$_{6}$–ptsI* (*B. subtilis*) under T5 promoter control in pQE30, *bla*, *ori* ColEI	(5)
pBGG190	*His$_{10}$–ptsN* under control of P_{tac}, *bla*, *ori* ColEI	(6)
pFDX283	P_{tac}, *tet*, *ori* p15A (empty vector)	(7)
pFDX2942	P_{tac}-*bglG*, *SD* of *gene10* of phage *T7* in front of *bglG*, *tet*, *ori* p15A	(3)
pFDX3158	*lacI^{q}*, P_{16}-*bglt2- lacZ*, *cat*, *ori* ColEI	(3)
pFDX3161	*ptsI* under control of P_{tac}, *tet*, *ori* p15A	(3)
pFDX3214	*fruB* under control of P_{tac}, *tet*, *ori* p15A	(3)
pFDX3221	*fruB and ptsI* under control of P_{tac}, *tet*, *ori* p15A	(3)
pFDX3225	P_{tac}-*galK′-bglG*, *SD* of *gene10* of phage *T7* in front of *galK′-bglG*, *tet*, *ori* p15A	(3)
pFDX3851	*ptsH and ptsI* under control of P_{tac}, *tet*, *ori* p15A	(4)
pFDX3852	*ptsH*-H15A *and ptsI* under control of P_{tac}, *tet*, *ori* p15A	(4)
pFDX4221	*bglG*-H208A and *bglF* under control of P_{tac}, *tet*, *ori* p15A	(8)
pFDX4291	operator-less P_{tac}, *sacB-SD*, MCS, *cat*, *ori* pSC101	(9)
pFDX4732	*fruB*, *ptsH*-H15A *and ptsI* under control of P_{tac}, *tet*, *ori* p15A	This work
pFDX4733	*ptsI* under control of P_{tac}, *cat*, *ori* pSC101	This work
pFDX4735	*ptsH* and *ptsI* under control of P_{tac}, *cat*, *ori* pSC101	This work
pFDX4736	*ptsH-H15A* and *ptsI* under control of P_{tac}, *cat*, *ori* pSC101	This work
pFDX4737	*fruB* and *ptsI* under control of P_{tac}, *cat*, *ori* pSC101	This work
pFDX4738	*fruB*, *ptsH-H15A* and *ptsI* under control of P_{tac}, *cat*, *ori* pSC101	This work
pFDX4739	*fruB* under control of P_{tac}, *cat*, *ori* pSC101	This work
pFDY226	*lacI^{q}*, P_{16}-*bglt2- lacZ*, *bla*, *ori* ColEI	(2)
pGP172	*T7*-promoter-*streptag*-MCS, *bla*, *ori* ColEI	(10)
pGP438	*strep-ptsH* (*B. subtilis*) under *T7* promoter control, *bla*, *ori* ColEI	S. Hübner
pGP1286	*strep-bglG* under *T7* promoter control, *bla*, *ori* ColEI	This work
pGP1287	*His$_{10}$–ptsH* (*E. coli*) under control of P_{tac}, *bla*, *ori* ColEI	This work
pGP1288	*His$_{10}$–fruB* under control of P_{tac}, *bla*, *ori* ColEI	This work
pGP1299	*His$_{10}$–ptsI* (*E. coli*) under control of P_{tac}, *bla*, *ori* ColEI	This work
pGP1309	as pGP1286, but bglG with a H208A exchange (CAT→GCT)	This work
pGP1319	*strep-s$_{tag}$-bglG* under *T7* promoter control, *bla*, *ori* ColEI	This work
pGP1320	*strep-s$_{tag}$-bglG*-H208A under *T7* promoter control, *bla*, *ori* ColEI	This work

Ori, origin of replication; *SD*, Shine-Dalgarno sequence; MCS, multiple cloning site; *strep*, sequence encoding the Strep-tag epitope, which is a synthetic peptide that exhibits intrinsic affinity toward the streptavidin derivative Strep-Tactin and consists of the eight amino acids Trp-Ser-His-Pro-Gln-Phe-Glu-Lys; s_{tag}, sequence encoding the S-tag epitope, which corresponds to the first 15 residues of pancreatic RNase A and consists of the amino acids Lys-Glu-Thr-Ala-Ala-Ala-Lys-Phe-Glu-Arg-Gln-His-Met-Asp-Ser.

1. Parche S, Schmid R, Titgemeyer F (1999) The phosphotransferase system (PTS) of *Streptomyces coelicolor* identification and biochemical analysis of a histidine phosphocarrier protein HPr encoded by the gene *ptsH*. *Eur J Biochem* 265:308–317.
2. Schnetz K, et al. (1996) LicT, a *Bacillus subtilis* transcriptional antiterminator protein of the BglG family. *J Bacteriol* 178:1971–1979.
3. Görke B, Rak B (1999) Catabolite control of *Escherichia coli* regulatory protein BglG activity by antagonistically acting phosphorylations. *EMBO J* 18:3370–3379.
4. Reichenbach B, Breustedt DA, Stülke J, Rak B, Görke B (2007) Genetic dissection of specificity determinants in the interaction of HPr with enzymes II of the bacterial phosphoenolpyruvate:sugar phosphotransferase system in *Escherichia coli*. *J Bacteriol* 189:4603–4613.
5. Galinier A, et al. (1997) The *Bacillus subtilis crh* gene encodes a HPr-like protein involved in carbon catabolite repression. *Proc Natl Acad Sci USA* 94:8439–8444.
6. Lüttmann D, et al. (2009) Stimulation of the potassium sensor KdpD kinase activity by interaction with the phosphotransferase protein IIA$^{(Ntr)}$ in *Escherichia coli*. *Mol Microbiol* 72: 978–994.
7. Görke B, Rak B (2001) Efficient transcriptional antitermination from the *Escherichia coli* cytoplasmic membrane. *J Mol Biol* 308:131–145.
8. Görke B (2003) Regulation of the *Escherichia coli* antiterminator protein BglG by phosphorylation at multiple sites and evidence for transfer of phosphoryl groups between monomers. *J Biol Chem* 278:46219–46229.
9. Kalamorz F, Reichenbach B, März W, Rak B, Görke B (2007) Feedback control of glucosamine-6-phosphate synthase GlmS expression depends on the small RNA GlmZ and involves the novel protein YhbJ in *Escherichia coli*. *Mol Microbiol* 65:1518–1533.
10. Merzbacher M, Detsch C, Hillen W, Stülke J (2004) *Mycoplasma pneumoniae* HPr kinase/phosphorylase. *Eur J Biochem* 271:367–374.

Table S2. Oligonucleotides used in this study

Primer	Sequence	Res. sites	Purpose
FR108	AAAGAGCTCGatgAACATGCAAATCACCAAAATTCTC	SacI	*bglG* forward
FR109	TTTGGATCCtcaGTGTTCTTTGCGCACG	BamHI	*bglG* reverse
FR120	AAAGCTAGCatgTTCCAGCAAGAAGTTACCATTAC	NheI	$ptsH_{Ec}$ forward
FR121	TTTTCTAGAttaCTCGAGTTCCGCCATCA	XbaI	$ptsH_{Ec}$ reverse
FR122	AAAGCTAGCatgTTCCAGTTATCCGTACAGG	NheI	*fruB* forward
FR123	TTTTCTAGAttaTGCGCCCTCCCCAAG	XbaI	*fruB* reverse
FR126	GCACAGCTGAACCTGAAG		$ptsI_{Ec}$ sequencing
FR127	GCGCAGTTCCTGTTTGTAG		$ptsI_{Ec}$ sequencing
FR128	AAAGCTAGCatgATTTCAGGCATTTTAGCATCCCCGGGTATC	NheI	$ptsI_{Ec}$ forward
FR129	TTTTCTAGAttaGCAGATTGTTTTTTCTTCAATGAACTTGTTAACCAG	XbaI	$ptsI_{Ec}$ reverse
FR181	AAAGAGCTCGAAAGAAACCGCGGCGAAATTTGAACGTCAGCATA TGGATAGCatgAACATGCAAATCACCAAAATTCTC	SacI	$bglG\text{-}S_{tag}$ forward
SB46	AAAGGATCCttaCTCGCCGAGTCCTTCGCTTTTCAT	BamHI	$ptsH_{Bs}$ reverse
SHU54	AAAGAGCTCgatgGCACAAAAAACATTTAAAGTAACTGC	SacI	$ptsH_{Bs}$ forward

Restriction sites are underlined; start and stop codons are in small caps. *Ec*, *E. coli*; *Bs*, *B. subtilis*.