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Most of us feel, from time to time, that other authors have not acknowledged the work of our own or other groups or have omitted to interpret important aspects of their own data. Perhaps we have observations that, although not sufficient to merit a full paper, add a further dimension to one published by others. In other instances we may have a useful piece of methodology that we would like to share.

The Editors hope that readers will take full advantage of this section and use it to raise matters that hitherto have been confined to a limited audience.

Jon Saunders, Editor-in-Chief

A hybrid response regulator possessing a PEP-dependent phosphorylation domain

The phosphoenolpyruvate (PEP):sugar phosphotransferase system (PTS) is a modular multiprotein phosphorelay system that mediates the concomitant detection, transport and phosphorylation of its sugar substrates (10). By virtue of its role as a protein kinase system, the PTS also regulates a variety of metabolic and transcriptional processes (19). Recent studies have revealed PTS-dependent phosphorylation mechanisms that play a direct role in transcriptional control. For example, the PTS serves to phosphorylate and modulate the activities of specific multidomain transcriptional activators and antiterminators, DNA- and RNA-binding proteins that share homologous phosphorylation domains (1, 5, 20, 21).

In Gram-positive bacteria, carbon catabolite repression is mediated by a transcription factor, CcpA, which functions together with a PTS transcription cofactor, HPr, that can be phosphorylated either on histidine (His-15) at the expense of PEP or on serine

Fig. 1. Domain structure and conserved sequence elements in HPRR. The HPr-like module (HPr, ▨), the putative linker regions (L, ▢), the two tandem PAS-containing domain (PAS, ▤), the σ^N activation central domain (C, ▥) and the C-terminal helix–turn–helix- (HTH, ▧) containing domain (▩) in the *C. acetobutylicum* HPRR are shown in the top line. HPr and the NtrC response regulator of *E. coli* are portrayed in the bottom line. A Q-linker (Q, ▦) separates the receiver module (R, ▨) from the central domain (C, ▥) of NtrC. The positions of the amino acids in the primary sequences that delineate the distinct domains of HPRR are indicated in the top line. Also shown are the positions of the catalytic His and the regulatory Ser in the HPr-like domain of HPRR and in HPr, the phosphorylated Asp in the receiver module of NtrC and the two putative ATP-binding sites in the two homologous activation domains.

(Ser-46 in most Gram-positive HPrs) at the expense of ATP. The former reaction is catalysed by Enzyme I of the PTS while the latter is catalysed by a metabolite-activated HPr(Ser) kinase (3, 12, 18). The two dissimilar phosphorylation reactions are mutually antagonistic and they exert opposing effects on transcription (12, 13, 15). These phosphorylation-dependent regulatory mechanisms are the only currently documented cases in which the PTS functions directly in transcriptional control.

A distinct modular multidomain transcriptional regulatory device, the so-called 'two-component sensor kinase/response regulator system,' mediates responses to diverse environmental signals through changes in gene expression and cell behaviour (8, 17). Minimally, these signal transduction pathways consist of a sensor kinase that senses an environmental stimulus and catalyses autophosphorylation of a His residue at the expense of ATP, and a response regulator that is phosphorylated by the kinase on an Asp residue and effects a cellular response, usually by interacting with a transcriptional control element in the DNA.

In this communication we describe a novel modular multidomain protein from *Clostridium acetobutylicum* that combines elements derived from the PTS and response regulators. While this protein exhibits sequence similarity to the NtrC family of response regulators in its two C-terminal domains (the ATP-hydrolysing/ σ^N -binding domain and the helix–turn–helix DNA-binding domain), it possesses an HPr-like domain instead of the usual N-terminal receiver module (Fig. 1). We have named this protein the HPr-response regulator (HPRR).

While NtrC is usually phosphorylated by the NtrB histidine kinase in an ATP-dependent reaction, we propose here that the Enzyme I-catalysed PEP-dependent phosphorylation of HPRR controls the transcription-enhancing activity of its effector domains. Accordingly, Enzyme I and HPRR may be analogous to sensor kinases and response regulators, respectively, of classical two-component systems.

HPRR (667 aa) is encoded in a monocistronic operon in *C. acetobutylicum* ATCC 824 (contig 1821). Its identification was made possible by analyses of the genome sequence of this organism (<http://www.cric.com/gene/sequences/clostridium/clospage.html>). It represents the first example of a putative

► GUIDELINES

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response regulator in which PEP can serve as the phosphoryl donor and a PTS constituent can function as the sensor.

Fig. 1. shows the modular structure of HPRR. The HPr-like domain (84 aa) is found at the extreme N terminus of the protein. This is followed by 169 residues that exhibit significant similarity (28 % identity throughout its length; 21sd) to an equivalent region in a putative σ^N - (the σ^N equivalent in *Bacillus subtilis*) dependent transcriptional regulator of *Bacillus subtilis* (SWISS-PROT P54529). It also exhibits limited similarity (1–9sd) to FixL of *Rhizobium leguminosarum* and a number of phytochromes and putative sensor kinases. A search of the profile entries in PROSITE (2) revealed that this region contains two tandem PAS domains. PAS domains are usually associated with oxygen- and light-sensing proteins as well as sensor kinases that function in signal transduction (4, 6, 9, 22). The dual PAS-containing sequence in HPRR is flanked by short regions (32 and 72 aa) which may serve as interdomain linkers. The third and the fourth recognizable modules in HPRR exhibit sequence similarity to the ATP-hydrolysing/ σ^N -binding domain and the helix–turn–helix-containing DNA-binding domain in the NtrC family of σ^N enhancer-binding proteins (7).

Partial sequences of the HPr-like domain, the σ^N -activating domain and the DNA-binding motif of HPRR are aligned with their homologous counterparts of known function in Fig. 2(a-c). In Fig. 2(a), a portion of the HPr-like domain in HPRR is aligned with the corresponding region in the *Escherichia coli* HPr. These sequences exhibit 29% identity with a comparison score of 9 SD. Phylogenetic tree construction revealed that the HPr-like domain in HPRR is distantly related to all other currently sequenced HPrs and HPr-like domains (data not shown). It nevertheless possesses the two phosphorylation residues of characterized HPrs, His-15 (the target of Enzyme I phosphorylation) and Ser-51 [the equivalent of Ser-46, the target of HPr(Ser) kinase phosphorylation in HPrs of Gram-positive bacteria]. The HPr-like domain of HPRR probably does not substitute for HPr in the phosphorylation cascade which culminates in sugar translocation, since a monocistronic operon bearing the *ptsH* gene encoding HPr is found elsewhere on the *C. acetobutylicum* chromosome.

Fig. 2(b) compares a portion of the ATP-hydrolysing/ σ^N -binding domain in HPRR with the homologous domain in *E. coli* NtrC. The degree of sequence identity observed between HPRR and NtrC is 52% with a comparison score of 81 SD. The alignment of the C-terminal helix-turn-helix DNA-binding region of HPRR with the helix-turn-helix motif of NtrC is shown in Fig. 2(c). Unpublished observations show that Leu-8 and Gly-9 are the only residues conserved in the helix-turn-helix motif of HPRR and the

majority of activators comprising this family.

The nature of the domains that comprise HPRR (Figs 1 and 2) suggests that this protein is a transcriptional activator of a gene or genes transcribed by RNA polymerase holoenzyme containing the alternative σ factor, σ^N . Divergently transcribed and upstream of the HPRR-encoding gene is a monocistronic operon encoding Enzyme I. This *ptsI* gene is preceded by a characteristic -12/-24 σ^N promoter (5'-TGGCAT-N₅-TTGCT-3'). In contrast to the multiplicity of Enzyme I-like proteins in *E. coli* (17), no additional PTS paralogue of Enzyme I appears to be encoded within the *C. acetobutylicum* genome. Consequently, it is likely that σ^N -dependent transcription of *ptsI* in this organism is regulated by PEP-dependent, Enzyme I-catalysed phosphorylation of HPRR. These observations suggest a novel type of autoregulatory mechanism, and since phosphorylation of Enzyme I is the first committed step in the phosphorylation cascade that culminates in PTS-dependent sugar translocation, they also imply that sugar translocation in *C. acetobutylicum* is at least in part under the transcriptional control of σ^N . The HPRR-mediated control of *ptsI* expression could in turn effect coordination of PTS-mediated sugar uptake with nitrogen metabolism as proposed previously (11,14,16).

HPRR described here provides a novel example of (1) a transcriptional regulator that is probably phosphorylated by the PTS; (2) a link between the PTS and so-called 'two-component' regulatory systems, two principal bacterial protein phosphorylating systems, and (3) a protein that must have evolved by splicing together modules from unrelated ancestral genes. The discovery of its occurrence in *C. acetobutylicum* relates to our earlier suggestion that PTS-catalysed protein phosphorylation regulates transcription of σ^N -dependent operons in *E. coli* (11, 14, 16). Experiments are currently in progress to

determine the precise function and mechanism of action of HPRR.

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Fig. 2. Alignment of sequence elements in HPRR with those of HPr and the response regulator, NtrC. (a) Alignment of a portion of the HPr-like domain of the *C. acetobutylicum* HPRR with a portion of the HPr of *E. coli*. In (a) and (b), conserved residues are indicated with an exclamation mark. The active site His residue and the phosphorylated regulatory Ser residue are highlighted (black background). (b) Alignment of a portion of the activator domain of HPRR with the homologous domains of the *E. coli* NtrC. The putative ATP-binding sequences in HPRR are underlined. (c) Helix–turn–helix motif of HPRR and NtrC of *E. coli*. Residues conserved in the majority of the NtrC-like activators of the σ^{54} -holoenzyme form of RNA polymerase are highlighted (black background).

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