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Minireview

Cross-talk of global nutritional regulators in the control of primary and secondary metabolism in *Streptomyces*

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Summary

Limitation of different nutrients in *Streptomyces coelicolor* A3(2) triggers nutrient-stress responses, mediated by PhoP, GlnR, AfsR and other regulators, that are integrated at the molecular level and control secondary metabolite biosynthesis and differentiation. In addition, utilization of chitin or *N*-acetylglucosamine regulates secondary metabolite biosynthesis by a mechanism mediated by DasR. Phosphate control of primary and secondary metabolism in *Streptomyces* species is mediated by the two-component PhoR–PhoP system. In *S. coelicolor*, PhoP controls secondary metabolism by binding to a PHO box in the *afsS* promoter overlapping with the AfsR binding site. Therefore, the *afsS* promoter serves to integrate the PhoP-mediated response to phosphate limitation and AfsR-mediated responses to other unknown environmental stimuli. Interestingly, phosphate control oversees nitrogen regulation but not vice versa. In Δ*phoP* mutants, expression of some nitrogen metabolism genes including *glnA*, *glnII* and *glnK* is increased. Phosphate control of these genes is exerted through binding of PhoP to the promoters of *glnR* (the global nitrogen regulator), *glnA*, *glnII* and the *amtB–glnK–glnD* operon. This regulation allows a 'metabolic homeostasis' of phosphate and nitrogen utilization pathways, preventing nutritional unbalances. Similar mechanisms of interaction between phosphate control and carbon catabolite regulation or between phosphate and DasR-mediated *N*-acetylglucosamine regulation appear to exist. Transport of *N*-acetylglucosamine by the NagE2 permease and, therefore, regulation of secondary metabolism, is dependent upon the balance of phosphorylated/dephosphorylated proteins of the *N*-acetylglucosamine phosphotransferase system. These findings provide the bases for understanding the mechanisms underlying systems biology of *Streptomyces* species.

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Introduction

Interaction of global regulators: a novel aspect of systems biology in streptomycetes

Extracellular environmental signals (e.g. pH stress or heat shock) and nutrient limitation signals are transduced through global regulators that in turn activate pathway-specific regulators (Rigali *et al*., 2008; Martín and Liras, 2010). During the past decade important advances have been made in our understanding of the mechanisms of control of primary and secondary metabolism in *Streptomyces* species by the limitation of different nutrients including (i) phosphate (Sola-Landa *et al*., 2003; 2008; Martín, 2004; Rodríguez-García *et al*., 2007; 2009), (ii) easily utilized nitrogen sources (e.g. ammonium) (Wray *et al*., 1991; Wray and Fisher, 1993; Fink *et al*., 2002; Hesketh *et al*., 2002; Tiffert *et al*., 2008), and (iii) glucose or other easily utilized carbon sources (the so-called carbon catabolite regulation) (Borodina *et al*., 2008). There is also an interesting mechanism of regulation of the *Streptomyces coelicolor* metabolism by *N*-acetylglucosamine (Rigali *et al*., 2008).

Each of these mechanisms is mediated by a global regulator that may be a member of a two-component system or an orphan global regulator. Phosphate control is mediated by the PhoR–PhoP system (Sola-Landa *et al*., 2003; 2005); nitrogen metabolism is controlled by the orphan response regulator, GlnR (and perhaps GlnRII; see below) (Wray *et al*., 1991; Wray and Fisher, 1993), and the chitin degradation and *N*-acetylglucosamine metabolism is mediated by a member of the

GntR family (Rigali *et al*., 2008). We will not describe in detail these regulatory mechanisms in this minireview since they have been reviewed previously (Martín, 2004; Rigali *et al*., 2008; Tiffert *et al*., 2008).

Our interest concentrates on the interactions between nutritional global regulators, a subject that has emerged in the last few years as a promising field to understand the coordination of metabolism and the response to different nutritional factors in *Streptomyces* (Martín and Liras, 2010). The interaction between global regulators described below allows the cells to maintain an equilibrium of nutritional pathways (nutrient homeostasis), an aspect of great interest in natural conditions in the soil where feast and famine episodes are frequent. Similar coordination mechanisms have been described in other bacteria particularly in *Escherichia coli* and *Bacillus subtilis* (Kasahara *et al*., 1991; Commichau *et al*., 2006; Oh *et al*., 2007; Sonenshein, 2007).

Genes controlled by PhoP: an overview

Phosphate has essential metabolic, structural and regulatory roles in *Streptomyces* species and other microorganisms (Rao and Torriani, 1990; Torriani, 1990; Wanner, 1996). Phosphate-containing metabolites take part in the central and energy metabolism, in the synthesis of membrane lipids and nucleic acids, and in the post-translational regulation of many proteins via phosphorylation (Wanner, 1996; Parker *et al*., 2010). In several *Streptomyces* species phosphate control is mediated by the two-component system PhoR–PhoP (Sola-Landa *et al*., 2003; 2005; Ghorbel *et al*., 2006a; Mendes *et al*., 2007). Genes under direct control of PhoP constitute, by definition, the *pho* regulon. In the last decade considerable progress has been made in our understanding of the mechanisms of phosphate control of primary and secondary metabolism in *Streptomyces* species. Several PhoP operators have been characterized by protein–DNA binding assays (EMSA and DNase I footprinting) (Apel *et al*., 2007; Rodríguez-García *et al*., 2007; 2009; Sola-Landa *et al*., 2005; 2008; Santos-Beneit *et al*., 2008; 2009a,b). In many promoters, PhoP works as a positive regulator, by binding at the −35 region (or nearby), but in some cases it may act as a repressor, when bound to the −10 promoter region (i.e. as a road-block for the RNA polymerase) (Apel *et al*., 2007).

The total number of genes responding to Pi limitation is considerably larger than those directly regulated by PhoP, as concluded from the transcriptome and proteome analysis of *S. coelicolor* wild-type and Δ*phoP* mutant strains after Pi depletion (Rodríguez-García *et al*., 2007). This is probably due to a PhoP-independent phosphate control mechanism that is still largely unknown. New interesting aspects of the interaction of PhoP with other regulators is becoming of paramount importance to understand the mechanisms of the transition from primary to secondary metabolism.

Response to phosphate starvation

Inorganic phosphate affects many reactions of primary and secondary metabolism in the cell. Therefore starvation of inorganic phosphate triggers important changes in the cell metabolism (Martín, 2004). These changes are of particular relevance in the transition from primary to secondary metabolism (Nieselt *et al*., 2010). Organic phosphates are abundant in plant and animal decaying materials but they are more difficult to be utilized by microorganisms than inorganic phosphate. However, most *Streptomyces* species have adequate phosphatase systems for efficient hydrolysis of the organic phosphates.

The primary response to Pi starvation involves the induction of extracellular enzymes to obtain Pi from organic phosphates, and the activation of genes encoding Pi transporters. Several phosphatases are activated in streptomycetes in response to phosphate starvation. The main organic phosphorus compounds in plant and animal-decaying materials in soil are phospholipids, inositol phosphates, sugar phosphates and nucleic acids. Inositol phosphates (phytates), which serve as phosphorous storage compounds in plants, frequently represent up to 80% of the total soil organic phosphorous (Quiquampoix and Mousain, 2005). The *phoA* and *phoD* genes encoding the secreted alkaline phosphatase and phospholipase D proteins, respectively, are activated by PhoP (Apel *et al*., 2007). The alkaline phosphatase (encoded by *phoA*) is a non-specific phosphomonoesterase which is commonly induced in response to Pi starvation in bacteria (Wanner, 1996). This enzyme was purified to homogeneity from *Streptomyces griseus* and its N-terminal amino acid sequence was used to identify the gene (Moura *et al*., 2001). In addition, the *S. coelicolor* genome contains another alkaline phosphatase gene, *phoC*. This gene, which shows a poor expression level, is induced by low Pi concentration in a PhoP-independent manner (Apel *et al*., 2007).

Another important group of enzymes that respond to phosphate starvation are phospholipases which release phosphatidic acids from phospholipids. Phospholipases are well-known virulence factors; they participate in Pi scavenging in both pathogenic and non-pathogenic bacteria (Titball, 1993; Oh *et al*., 2007). Several phospholipase D genes have been sequenced because of their biotechnological applications including genes from *Streptomyces griseofuscus* and *Streptomyces antibioticus* (Iwasaki *et al*., 1994; Masayama *et al*., 2008; Uesugi and Hatanaka, 2009).

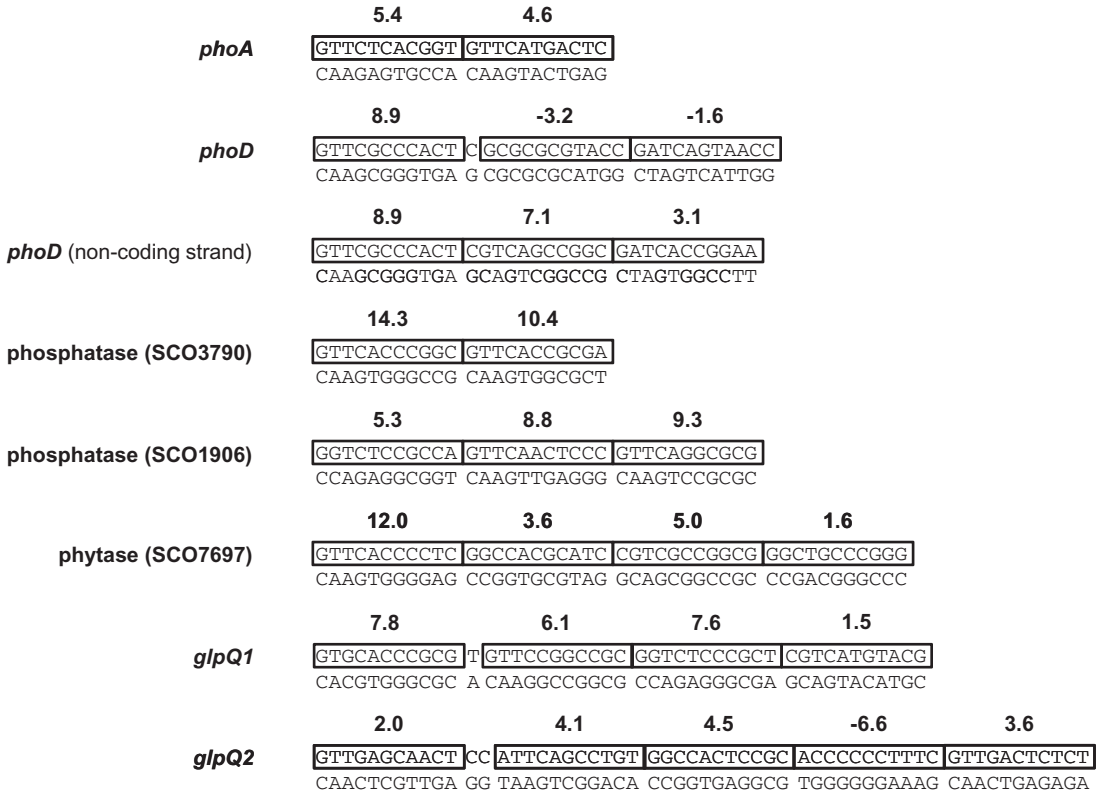

Fig. 1. Sequences of the PHO boxes present in the promoters of the alkaline phosphatases *phoA* and *phoD*, other two putative phosphatases (*SCO1906* and *SCO3790*), a putative phytase (Apel *et al*., 2007; Sola-Landa *et al*., 2008) and two *glpQ* genes (Santos-Beneit *et al*., 2009b). The DRus are boxed, and the individual information (*R*i) for each one is indicated. This value was calculated using the Model 2 of PhoP operators (A. Rodríguez-García, unpublished).

At least other three secreted enzymes are encoded by *pho* regulon genes. These are the glycerophosphodiester phosphodiesterases (GDPDs) encoded by *glpQ1* and *glpQ2* (Santos-Beneit *et al*., 2009b) and the putative phytase gene *SCO7697* (Rodríguez-García *et al*., 2007; Sola-Landa *et al*., 2008). Glycerophosphodiesters are released from phospholipids by deacylating phospholipases, and then are hydrolysed by GDPDs to yield sn-glycerol-3-phosphate and an alcohol (Larson *et al*., 1983). Specific transporters for the uptake of glycerol-3-phosphate have been characterized in Gram-positive and Gram-negative bacteria (Brzoska and Boos, 1988; Overduin *et al*., 1988; Nilsson *et al*., 1994). It is expected that homologous transporters exist in the *S. coelicolor* genome (A. Rodríguez-García, unpublished).

Since phytates are important sources of Pi, it is interesting that PhoP activates phytase genes in response to phosphate starvation in *Streptomyces* species (Rodríguez-García *et al*., 2007; Sola-Landa *et al*., 2008) as occurs also in *Bacillus licheniformis* and *Bacillus amyloliquefaciens* (Hoi *et al*., 2006; Makarewicz *et al*., 2006).

Other genes encoding putative phosphate scavengers are *SCO1906*, *SCO3790* and *SCO4152*. Both *SCO1906* and *SCO3790* encode putative secreted phosphatases (other than PhoA and PhoC) and their promoters are bound by PhoP (Rodríguez-García *et al*., 2007; Sola-Landa *et al*., 2008) (Fig. 1). *SCO4152* encodes a putative secreted 5′-nucleotidase that is upregulated in the wild-type transcriptome after phosphate shift-down, but not in a Δ*phoP* mutant. Therefore, this gene is a candidate member of the *pho* regulon.

Phosphate uptake

The released Pi from organic sources can enter the cell by means of (at least) three Pi transporters including the high-affinity Pst system, and the low-affinity high-velocity PitH1 and PitH2 systems (Díaz *et al*., 2005; Sola-Landa *et al*., 2005; Santos-Beneit *et al*., 2008). The *pitH1* promoter is active in the Δ*phoP* mutant, whereas the promoters of the *pstS* gene (the first gene of the *pstSCAB* cluster) and of the *pitH2* gene are totally dependent upon PhoP binding to become active. Following Pi depletion the highest activation of the *pitH2* promoter precedes that of the *pstS* promoter. The high-affinity transporter (Pst) is energetically more expensive to the cell (because it consumes ATP) than the low-affinity Pit systems which is driven by the proton motive force. Nevertheless, the Pst

Table 1. Metabolic responses to phosphate starvation.

Metabolic classes	Representative genes	Encoded proteins	References
Saving phosphate and using storage material	SCO4145 *ppk*[a]	Polyphosphate kinase	Ghorbel *et al.* (2006b) Nieselt *et al.* (2010)
	SCO4872-4882[a]	Genes for biosynthesis of a phosphate-free cell wall polymer	Rodríguez-García *et al.* (2007) Nieselt *et al.* (2010)
Modulation of central pathways	SCO7630 and SCO4209	Two paralogous phosphoglycerate mutases	Rodríguez-García *et al.* (2007)
Energy metabolism[b]	SCO2150-48 *qcrCAB* SCO2156-55 *cox2-1* SCO0216-17 *narG2-H2* SCO6534-35 *narH-G* SCO4947/50 *narG3/I3*	Downregulators of respiratory metabolism and upregulators of the nitrate reductase F0F1-ATP synthetase operon	Rodríguez-García *et al.* (2007) Nieselt *et al.* (2010)
Protein synthesis	Several operons[c]	Most ribosomal protein genes	Rodríguez-García *et al.* (2007) Nieselt *et al.* (2010)
Interaction with nitrogen metabolism	*glnA*, *glnII*, *amtB*, *glnD*, *glnK*, *glnR*	Glutamine synthetase, ammonium transport and nitrogen metabolism regulators	Rodríguez-García *et al.* (2007) Nieselt *et al.* (2010)
Oxidative stress and iron metabolism	SCO0379 *catA* SCO2113 *bfr* SCO5032 *ahpC*	Catalase Bacterioferritin Alkyl hydroperoxide reductase	Rodríguez-García *et al.* (2007) Nieselt *et al.* (2010)
Biosynthesis of secondary metabolites	*act* genes *red* genes	Actinorhodin biosynthesis[d] Undecylprodigiosin biosynthesis	Santos-Beneit *et al.* (2009a) Nieselt *et al.* (2010)

a. All these genes are upregulated after Pi depletion and are PhoP-dependent.
b. Phosphate starvation appears to produce a PhoP-dependent switch in energy metabolism, downregulating the aerobic metabolism genes and upregulating the nitrate reductase.
c. Most genes encoding ribosomal proteins are downregulated after Pi depletion.
d. Production of undecylprodigiosin and actinorhodin takes place after Pi depletion (undecylprodigiosin is switched on a few hours earlier than actinorhodin).

system is effective in conditions of very low Pi concentrations due to its high affinity for Pi. These factors explain the sequential pattern of expression of these genes observed in flask cultures of *S. coelicolor* (Santos-Beneit *et al.*, 2008) and in fermenters (Nieselt *et al.*, 2010).

In addition to phosphate scavenging and release of Pi from organic phosphates, starvation of the cultures for phosphate triggers several other responses to phosphate starvation stress (Table 1).

Interaction of phosphate and carbon metabolism

Carbon and nitrogen sources, in addition to phosphate, play an important role in the regulation of primary and secondary metabolism in almost all bacteria including streptomycetes (Martín and Demain, 1980; Hobbs *et al.*, 1990; Merrick and Edwards, 1995). These different mechanisms of control have been widely studied in an individual form, but the connections existing between them are still poorly known. A few examples of genes regulated simultaneously by different nutritional sources in *Streptomyces* species have been described up to now. The *glpQ1* and *glpQ2* genes of *S. coelicolor* (see previous section) are directly regulated by PhoP (Rodríguez-García *et al.*, 2007; Santos-Beneit *et al.*, 2009b) and their expression is also affected, in a different manner, by the carbon source (Santos-Beneit *et al.*, 2009b).

An interaction of phosphate and carbon source regulation has been described for the *pstS* gene in *Streptomyces lividans* and *S. coelicolor*. PstS is the phosphate-binding protein belonging to the high-affinity phosphate-specific transport system (*pst*), a member of the *pho* regulon (Díaz *et al.*, 2005; Sola-Landa *et al.*, 2005; Rodríguez-García *et al.*, 2007). The extracellular PstS protein is accumulated in high concentrations in liquid cultures when the bacteria are grown in the presence of different sugars under phosphate limitation conditions (Díaz *et al.*, 2005; Esteban *et al.*, 2008). Phosphorylated intermediates of the glycolysis may serve as integrator molecules of the phosphate and carbon metabolism. An interaction between phosphate and carbon sources has been shown also in other microorganisms; in *Vibrio vulnificus*, the expression of alkaline phosphatase and phospholipase genes is affected by both phosphate concentration and the carbon source (Oh *et al.*, 2007). In *B. subtilis* it is known that the carbon catabolite protein-A (CcpA) exerts a control over the expression of *phoRP* genes, although there is a discrepancy about whether such regulation is exerted through the *cre* sequence (Puri-Taneja *et al.*, 2006) or indirectly (Choi and Saier, 2005). The cross-talk between CcpA and the PhoRP system is a good example of interaction between mechanisms controlling metabolic fluxes in bacteria. In *S. coelicolor* the integration of the carbon and phosphate signalling pathways may be mediated by phosphorylated glycolytic intermediates since the phosphofructokinase

gene (*pfkA2*) has been reported to play an important role in the control of secondary metabolite biosynthesis (Borodina *et al*., 2008).

Phosphate control oversees nitrogen regulation

The relationship between the phosphate and nitrogen regulatory pathways in *S. coelicolor* is complex because it affects not only the expression of genes involved in transport and assimilation of the nitrogen sources but also the direct nitrogen regulators. GlnR is the main nitrogen regulator and controls the expression of several genes involved in nitrogen metabolism at the transcriptional level (Wray and Fisher, 1993; Reuther and Wohlleben, 2007). In response to nitrogen limitation GlnR activates the transcription of numerous genes (Tiffert *et al*., 2008), including that of *glnA* and *glnII* (encoding two different types of glutamine synthetases) and the operon *amtB–glnK–glnD*, involved in ammonium transport and metabolism (Reuther and Wohlleben, 2007). GlnRII is a second regulator similar to GlnR that also binds to the promoters of *glnA*, *glnII* and *amtB* genes (Fink *et al*., 2002). However, GlnRII is not totally homologous to GlnR and its role in nitrogen regulation is not yet clear (Reuther and Wohlleben, 2007).

Recently, it was discovered that the regulation of nitrogen metabolism in *S. coelicolor* is under PhoP control (Rodríguez-García *et al*., 2007). Under phosphate limitation the expression of some nitrogen metabolism genes like *glnA*, *glnII*, *amtB* and *glnK* is increased in a *S. coelicolor* Δ*phoP* mutant (Fig. 2). The transcriptomic data clearly indicate that these genes are overexpressed in the Δ*phoP* mutant. The control of PhoP over these genes is exerted indirectly through binding of this regulator to the promoter of *glnR*, responsible for their activation. In addition, there is also a direct control exerted by the binding of PhoP to the promoters of *glnA*, *glnII* and the *amtB–glnK–glnD* operon (Rodríguez-García *et al*., 2009). In the regulation of *glnA* there is a direct competition between the PhoP and GlnR proteins, which recognize overlapping sequences in its promoter (Rodríguez-García *et al*., 2009) (Fig. 3). GlnR has a higher affinity *in vitro* for this region than PhoP (A. Sola-Landa, unpublished). Overlapping binding sequences occur also in the *glnII* promoter. In the *amtB* promoter the PhoP- and GlnR-binding sequences are not overlapping, but PhoP appears to act as a 'roadblock', preventing the correct formation of the RNA polymerase complex needed for the transcription (Fig. 3). This double mechanism (repression of *glnR* and also of the *amtB–glnD–glnK* operon) ensures a fine control of *glnA*, *glnII* and *amtB–glnK–glnD* by PhoP. A possible explanation for the phosphate–nitrogen control is the need for overall coordination of metabolism. Under phosphate limitation, the cells need to channel their energy resources to obtain phosphate and decrease the expression of the genes for nitrogen assimilation in order to equilibrate the P/N ratio in the hyphae, avoiding the energy expenses related to the utilization of unnecessary nitrogen sources.

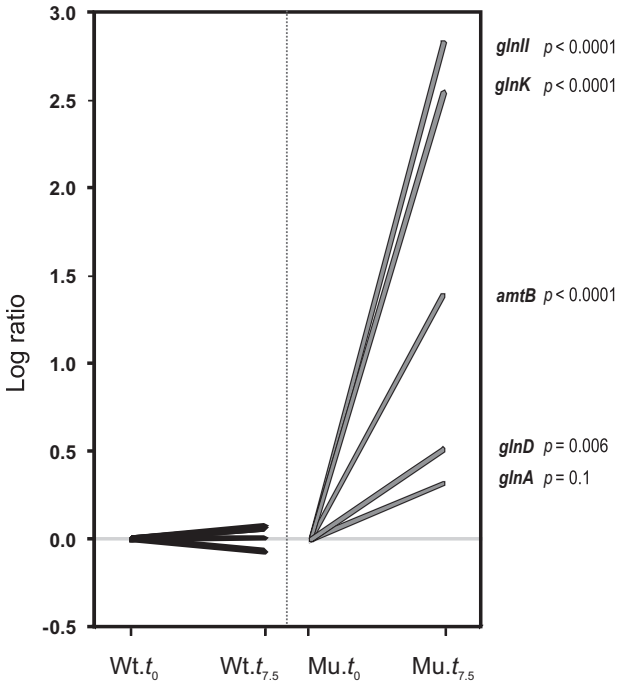

Fig. 2. Transcriptional responses (microarrays data) of several nitrogen metabolism genes (*glnA*, *glnD*, *amtB*, *glnK* and *glnII*) to phosphate starvation in the parental strain *S. coelicolor* M145 (left; black lines) and in the Δ*phoP* mutant (right; grey lines). The vertical axis indicates the transcription value calculated as the normalized log2 of Cy3/Cy5 intensities. The Cy3 fluorescent intensity corresponded to the labelled cDNA and the Cy5 signal to the labelled gDNA as the common reference. Wt, parental strain M145 at time 0 (phosphate-replete condition) and at 7.5 h after the phosphate shift-down. Mu, Δ*phoP* mutant at time 0 and at 7.5 h after shift-down. The data were obtained as described in Rodríguez-García and colleagues (2007).

Interestingly, PhoP binds to the promoter of *glnR* gene but not to the promoter of *glnRII*, suggesting that there is a 'second' nitrogen regulatory system independent of PhoP (Rodríguez-García *et al*., 2009). The cross-regulation between phosphate and nitrogen metabolism is not reciprocal since GlnR does not bind to promoters of genes of the *pho* regulon such as *pstS* and *phoRP* (A. Sola-Landa, unpubl. results).

Similar coordination mechanisms occur also in other microorganisms. A negative effect of phosphate starvation over the expression of nitrogen metabolism genes has been described in *E. coli* and *Sinorhizobium meliloti* (Van-Bogelen *et al*., 1996; Krol and Becker, 2004). It is very likely that similar phosphate–nitrogen interaction mechanisms will be discovered when systems biology studies are applied to other bacteria.

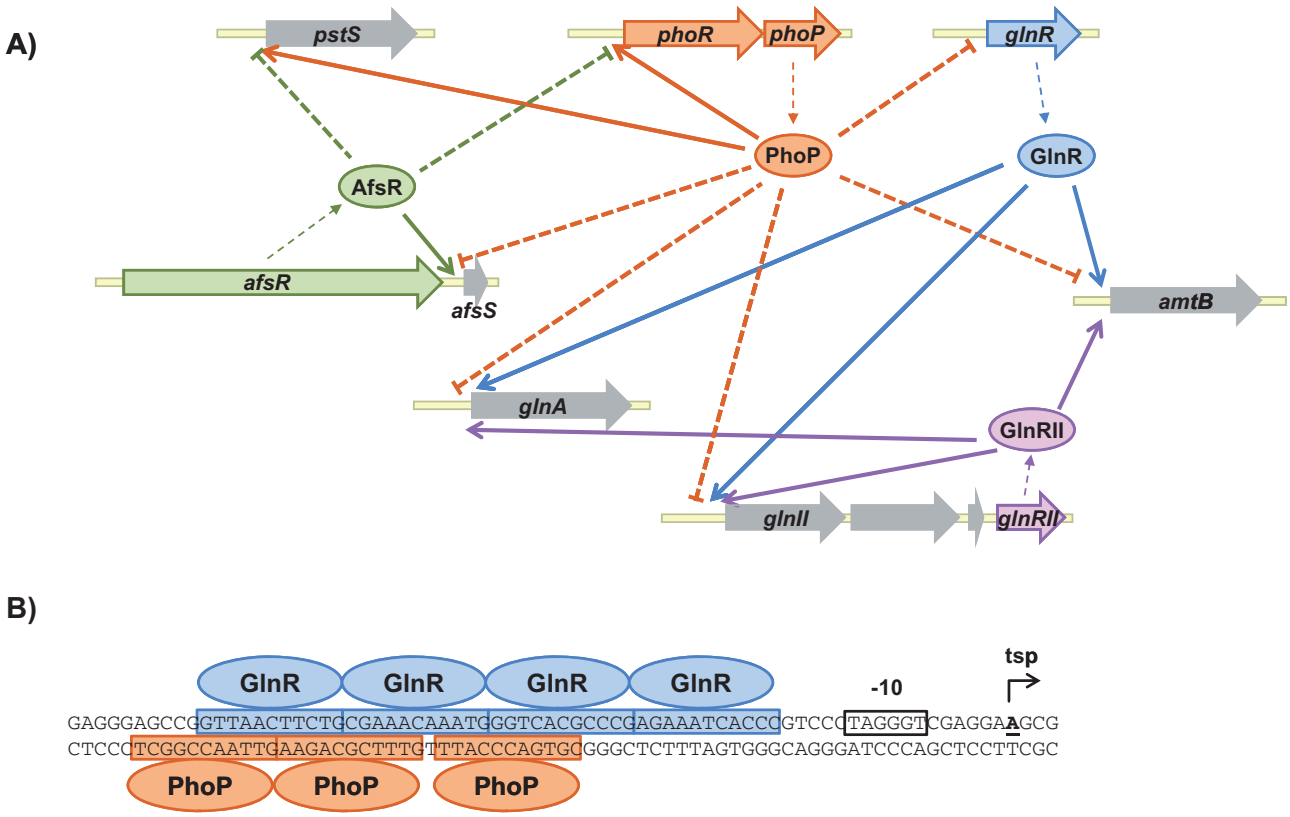

Fig. 3. A. Regulatory network of PhoP, GlnR, AfsR and GlnRII over the main genes involved in phosphate and nitrogen metabolism. Solid line arrows indicate induction whereas blunt-ended dashed lines indicate repression. Thin dashed arrows indicate translation of the regulators.
B. Regulation of PhoP and GlnR over *glnA* promoter. The 11 nt repeat sequences recognized by GlnR (according to Reuther and Wohlleben, 2007) in the coding strand are boxed, and the 11 nt repeat sequences recognized by PhoP (according to Rodríguez-García *et al*., 2009) are boxed and shaded. The –10 box and the transcription start point (tsp) are also shown (Fisher and Wray, 1989).

Interaction of global regulators PhoP and AfsR in the control of secondary metabolite biosynthesis

The complex regulation of secondary metabolites production in *Streptomyces* species and other bacteria is under the control of both global and pathway-specific regulators, which in turn respond to a variety of signals. As indicated above, phosphate limitation triggers the biosynthesis of hundreds of secondary metabolites in different *Streptomyces* species. The PhoR–PhoP system was shown to be involved in the regulation of actinorhodin and undecylprodigiosin production in *S. lividans* and *S. coelicolor* (Sola-Landa *et al*., 2003; Santos-Beneit *et al*., 2009a) and also in pimaricin production in *Streptomyces natalensis* (Mendes *et al*., 2007). The absence of PHO boxes and the lack of direct binding of PhoP to the genes encoding the antibiotic biosynthetic enzymes or the pathway-specific regulators, *actII*-ORF4 and *redD*, suggests that the PhoP regulatory effect may be exerted through signalling cascades involving intermediate regulatory genes or through interaction of PhoP with other regulators.

The AfsR action was initially studied in *S. griseus* and in *S. coelicolor* (Horinouchi *et al*., 1983). Recently we have described a cross-talk between the PhoP and AfsR proteins (Santos-Beneit *et al*., 2009a). AfsR is a positive regulator of actinorhodin and undecylprodigiosin in *S. coelicolor* and *S. lividans*. Floriano and Bibb (1996) found that the stimulatory effect of AfsR on antibiotic production is exerted through the activation of the pathway-specific regulatory genes *actII*-ORF4 and *redD*. The only known target of the AfsR regulator is a small gene located downstream of *afsR*, named *afsS* in *S. coelicolor* and *afsR2* in *S. lividans*. A similar gene has been identified in the genomes of *S. griseus* and other *Streptomyces* species. Amplification of *afsR2/afsS* on a high-copy-number plasmid confers overproduction of actinorhodin and undecylprodigiosin in both *S. coelicolor* and *S. lividans* (Vögtli *et al*., 1994; Matsumoto *et al*., 1995; Floriano and Bibb, 1996). The binding sequence of AfsR to the *afsS* promoter region was identified by Lee and colleagues (2002); our analysis of this sequence identified a putative PHO box overlapping with the AfsR-binding sequence, which suggests that both PhoP and AfsR might interact in the regulation of *afsS*. DNase I footprinting studies revealed a PhoP protected region of 26 nucleotides that overlaps with the AfsR recognition sequence. Binding experiments

indicated a competition between AfsR and PhoP; increasing concentrations of PhoP prevented formation of the AfsR–DNA complex (Santos-Beneit *et al*., 2009a).

Expression studies with *S. coelicolor* wild-type and Δ*phoP* strains using the reporter *luxAB* gene coupled to the *afsS* promoter showed that PhoP downregulates *afsS* expression probably by a competition with the AfsR activator. The binding site of PhoP in the *afsS* promoter overlaps the −35 region. The exact role of PhoP in the absence of AfsR is being investigated but we cannot exclude the possibility that in response to phosphate starvation PhoP itself activates *afsS* when AfsR is not present.

Interestingly, AfsR binds to other PhoP-regulated promoters including those of *pstS* (a member of the phosphate transport system) and *phoRP* (encoding the two-component system itself). Analysis of the AfsR- and PhoP-protected sequences in each of these promoters together with mutagenesis analyses of the overlapping sequences allowed us to distinguish some nucleotides in the AfsR-binding sequence different from the consensus PHO box (Santos-Beneit *et al*., 2009a; F. Santos-Beneit, unpublished).

The regulation of *afsS* by AfsR and PhoP allows the bacteria to integrate different signals (i.e. nutrients starvation) in the regulation of primary and secondary metabolism; in other words, the *afsS* promoter region integrates different signals, e.g. phosphate starvation through PhoP or the still unknown stress signal sensed by AfsR-associated sensor kinases. The same argument is true for the *phoRP* and *pstS* promoters that bind both PhoP and AfsR.

Regulation of secondary metabolite biosynthesis by *N*-acetylglucosamine and the global regulator DasR

An additional mechanism of nutritional control by *N*-acetylglucosamine occurs in *S. coelicolor* and probably in some other *Streptomyces* species, although it is unclear if this mechanism is universal in all streptomycetes. *N*-acetylglucosamine is the monomeric component of chitin, a constituent of cell wall of many filamentous fungi that is also present in the cuticle of insects and crustaceous. This polymer is utilized as carbon and nitrogen source by chitinases that have been characterized in different *Streptomyces* species (Schrempf, 2001; Kezuka *et al*., 2006).

N-acetylglucosamine induces the enzymes involved in its utilization, and also, increases the biosynthesis of actinorhodin and undecylprodigiosin in *S. coelicolor* (Rigali *et al*., 2008). This control is mediated by the global regulator DasR, a member of the GntR family (Colson *et al*., 2007; Rigali *et al*., 2008). A *dasR* mutant showed increased production of both actinorhodin and undecylprodigiosin suggesting that DasR regulates negatively the biosynthesis of these antibiotics. A DasR-responsive element was found in the upstream region of the *crr–pts1* operon encoding the phosphotransferase system (PTS) enzyme, IIA (IIA^{Crr}), which partially matched the consensus DasR-binding sequence (Colson *et al*., 2007). DasR-responsive elements were found in the upstream regions of *actII*-ORF4 and *redZ*, the transcriptional activators of the *act* and *red* gene clusters.

It is intriguing why chitin (or *N*-acetylglucosamine) utilization is so important in the biosynthesis of actinorhodin and undecylprodigiosin. It seems that detection and utilization of these carbon sources may serve to sense the presence of fungi in soil, triggering a 'competition stress' mechanism that will lead to production of secondary metabolites by *S. coelicolor*, some of which may have antifungal activity, or may serve as communication signals triggering differentiation.

The DasR-mediated regulation is triggered during *N*-acetylglucosamine transport into the cells

Transport of *N*-acetylglucosamine is the first step that triggers the DasR-mediated transcriptional response. The NagE2 permease of the *S. coelicolor* phosphotransferase system is the key protein acting as *N*-acetylglucosamine receptor (Nothaft *et al*., 2010). Uptake of *N*-acetylglucosamine requires a phosphoryl group transfer from phosphoenolpyruvate via the phosphotransferases EI, HPr and IIA^{Crr} to NagF, which in turn phosphorylates *N*-acetylglucosamine during transport by the NagE2 permease, as described previously in *Streptomyces olivaceoviridis* (Wang *et al*., 2002; Saito and Schrempf, 2004). Transcription of the *nagF* and *nagE2* genes is induced by *N*-acetylglucosamine.

The need of sequential phosphorylation (phosphorelay) of these enzymes and the supply of phosphoenolpyruvate connect the phosphorylation status of the cell (i.e. its energy charge) with the transport of *N*-acetylglucosamine and the induction of secondary metabolite biosynthesis. Further research is still needed on the role of DasR at a global regulator of secondary metabolism and its connection with the mechanism of classical carbon catabolite regulation exerted by glucose.

Conclusions and future outlook

Several examples of the interactions between the global nutritional regulators PhoP, GlnR, DasR, AfsR and other poorly known nutritional regulators occur in *S. coelicolor*. The interaction or competition between these global regulators allows the cell to reach a 'metabolic homeostasis' in response to nutritional unbalances in the soil. We have limited the scope of this minireview to those well-known examples but several other global regulators related to

nutrition, are known in *Streptomyces* species (e.g. DmdR, SoxR), which probably respond to other nutritional stresses or environmental stimuli. Other regulators of antibiotic production like AbsA2, CutS, AbaB, AbsB, RapA2 and AfsQ2 have been described in *Streptomyces* species (Horinouchi *et al*., 1983; Champness *et al*., 1992; Scheu *et al*., 1997; Price *et al*., 1999; Chang *et al*., 2005; Lu *et al*., 2007; Shu *et al*., 2009) and further research is needed on the possible interactions with the global regulators described in detail in this article. A detailed knowledge of the molecular interactions and the integration of signals on the promoters of specific 'integrator' regulatory genes (e.g. *afsS*) will allow us to get a better picture of the key regulatory interactions that serve as the basis of systems biology.

The evidence available suggests that the PhoR–PhoP system is present in all sequenced *Streptomyces* genomes (e.g. *Streptomyces ambofaciens*, *Streptomyces avermitilis*, *S. griseus*, *Streptomyces clavuligerus*, etc.); however, given the great diversity of *Streptomyces* species, it is likely that modifications of the interactions between global regulators occur in some *Streptomyces.*

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