

MINIREVIEW

Molecular Control of Expression of Penicillin Biosynthesis Genes in Fungi: Regulatory Proteins Interact with a Bidirectional Promoter Region

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Genes for the biosynthesis of secondary metabolites are usually arranged in clusters (15, 59) together with genes for resistance to the toxic action of secondary metabolites on the producer organisms (20) and sometimes with genes for biosynthesis of antibiotic precursors (54). The penicillin biosynthesis cluster consists of three genes *pcbAB*, *pcbC*, and *penDE* (58) and are arranged the same in *Penicillium chrysogenum*, *Aspergillus nidulans* (1), and *Penicillium nalgiovense* (51) (Fig. 1). The *pcb* genes encode enzymes involved in penicillin and cephalosporin biosynthesis, whereas *pen* genes are specific for the penicillin pathway. The *pcbAB* and *pcbC* genes are expressed from a 1.16-kb bidirectional promoter region in opposite directions (6, 7, 21, 63). The expression of these genes is subject to sophisticated controls by both nutritional and developmental factors (1, 8, 9, 55, 60).

Multiple DNA-binding proteins appear to bind to different regions of the *pcbAB-pcbC* bidirectional promoter. Proteins that interact with the *pcbAB-pcbC* intergenic control region have been found by DNA mobility shift and DNA footprinting assays (17, 29; J. F. Martín, K. Kosalková, A. T. Marcos, F. Fierro, F. J. Fernández, and S. Gutiérrez, Abstr. 8th Int. Symp. Genet. Ind. Microorg., p. 22, 1998). Penicillin biosynthesis is sensitive to nitrogen source repression; the global-acting regulatory protein NIT2 of *Neurospora crassa* binds strongly to a site in the bidirectional promoter containing two close GATA sequences (29). Glucose strongly represses penicillin biosynthesis by preventing expression of the three penicillin biosynthesis genes (26, 75); the glucose effect is not due to a decrease in pH and is not reversed by alkaline pHs (37). Glucose repression of penicillin biosynthesis does not seem to be exerted by the global carbon catabolite regulator CreA (86) but appears to be mediated by complex regulatory protein interactions (48). Cooperation between these DNA-binding proteins modulate penicillin gene expression. Differences in the interacting proteins may explain the widely different levels of penicillin production in *P. chrysogenum* and *A. nidulans*.

THE PENICILLIN GENE CLUSTER *pcbAB-pcbC-penDE* IS AMPLIFIED IN TANDEM REPEATS IN HIGH-LEVEL PENICILLIN-PRODUCING STRAINS

The three genes encoding δ -(L- α -aminoadipyl)-L-cysteinyl-D-valine (ACV) synthetase, isopenicillin N (IPN) synthase (cyclase), and IPN acyltransferase, named *pcbAB*, *pcbC*, and *penDE*, respectively (21, 83), are clustered in a 15-kb DNA region in all

known penicillin-producing fungi (51, 62, 81, 82) (Fig. 1). The conserved arrangement of the penicillin biosynthesis genes in different species is undoubtedly important for coordinated regulation of expression of the three genes (57). The three genes occur only in a few evolutionarily related fungal species (1, 36).

The penicillin biosynthesis genes of *P. chrysogenum* AS-P-78 are amplified in tandem repeats of a 106.5-kb DNA region (five or six copies) linked by conserved TTTACA sequences (31, 68). The wild-type strains *P. chrysogenum* NRRL 1951 and *Penicillium notatum* ATCC 9478 (Fleming's isolate) contain a single copy of the 106.5-kb region. This region is flanked by the same TTTACA hexanucleotide found between tandem repeats in strain AS-P-78. A penicillin-overproducing strain, *P. chrysogenum* E1, contains a large number of copies in tandem with a 57.9-kb DNA fragment (corresponding to the righthand side moiety of the 106.5-kb region that includes the three penicillin genes) linked by the same hexanucleotide (or its reverse complementary TGTAAG) sequence. The amplification has occurred within chromosome I, the largest *P. chrysogenum* chromosome, which is 11.0 Mb long in strain AS-P-78 (31).

Several mutants unable to produce penicillin have been shown to have a deletion of the penicillin gene cluster (33). The *P. chrysogenum npe-10* deletion mutant showed a deletion of 57.9 kb that corresponds exactly to the DNA fragment that is amplified in strain E1. The conserved hexanucleotide TTTACA sequence was reconstituted at the deletion site. The tandem reiterations and deletions appear to arise by mutation-induced site-specific recombination at the conserved hexanucleotide sequences (30). No other gene, either structural or regulatory, that may affect penicillin biosynthesis is present in the amplified DNA region that occurs in the high-level penicillin-producing strains (32, 36).

PacC-MEDIATED pH CONTROL OF PENICILLIN GENE EXPRESSION

A pH regulatory circuit which controls extracellular enzymes, permeases, and other cellular processes is known in *A. nidulans* and other fungi (13, 88). Espeso and coworkers (27) showed that transcription of the *A. nidulans* gene encoding isopenicillin N synthase is under the control of the pH regulatory system mediated by the PacC protein and proposed that external alkaline pH overrides carbon regulation. PacC is a transcriptional factor that contains three putative Cys₂His₂ zinc fingers (88). The intergenic *pcbAB-pcbC* region of *A. nidulans* contains at least five PacC binding sites GCCARG. However, the PacC binding sites did not correspond to the *cis*-acting region involved in carbon catabolite regulation (88).

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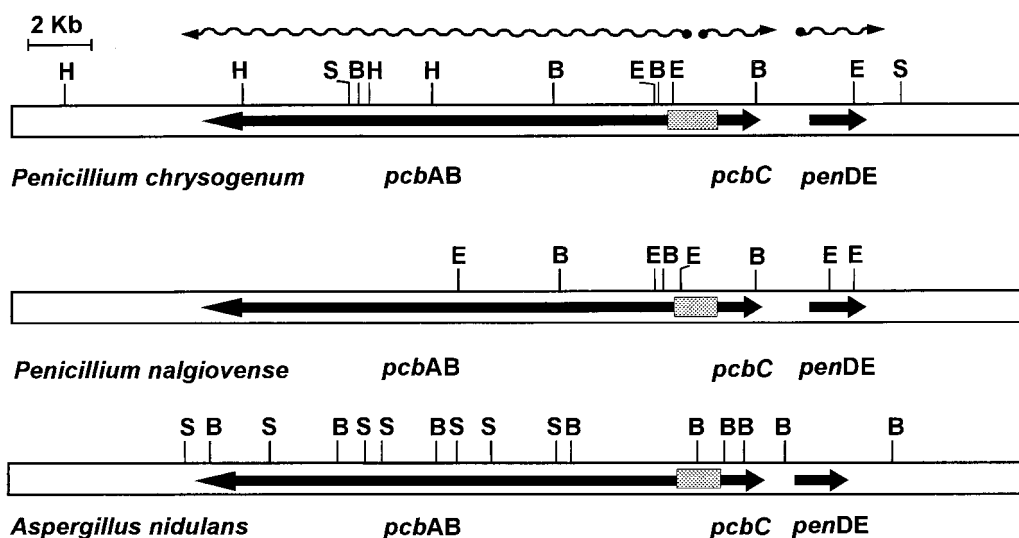


FIG. 1. Penicillin gene clusters in *P. chrysogenum*, *A. nidulans*, and *P. nalgiovense*. The *pcbAB*, *pcbC*, and *penDE* genes encode ACV synthetase, IPN synthase, and IPN acyltransferase, respectively. The arrows indicate the orientation of the genes. The bidirectional *pcbAB-pcbC* promoter region is stippled. The transcripts of the three genes are indicated by black wavy lines at the top of the figure. Restriction site abbreviations: S, *Sall*; B, *BamHI*; H, *HindIII*; E, *EcoRI*.

The effect of pH on regulation of penicillin biosynthesis was also observed in *P. chrysogenum* (18) although the effect of alkaline pH values seems to be weaker in this fungus than in *A. nidulans* (37). Alkaline pH values stimulate only slightly the expression of the penicillin biosynthesis genes, particularly of the *pcbC* and *penDE* genes (Fig. 2).

CARBON CATABOLITE REGULATION OF PENICILLIN BIOSYNTHESIS

Penicillin biosynthesis in *P. chrysogenum* is strongly regulated by glucose and sucrose and to a lower extent by other sugars (maltose, fructose, and galactose), but not by lactose (74). Lactose is the classically used carbon source for penicillin production, although continuous slow feeding of glucose, thus avoiding glucose repression, is frequently used in industrial processes.

High glucose concentrations prevent formation of ACV (the first intermediate of the pathway) and depress isopenicillin N synthase and (only to a low extent) acyl-coenzyme A:isopenicillin N acyltransferase (10, 75). Glucose-grown cultures showed reduced α -amino adipic acid pools (41), apparently caused by stimulating lysine biosynthesis and growth (75).

In *A. nidulans*, penicillin biosynthesis is repressed by sucrose and to a lower extent by glucose (10, 26). Some strains of *A. nidulans* (e.g., strain G191) seem to be less sensitive to glucose repression than others (46).

The repressive effect of glucose on penicillin biosynthesis is strongly dependent on the presence of inorganic phosphate in the culture medium. In a phosphate-limited complex medium (containing Pharmamedia as the only source of phosphate and nitrogen), the reduction in the penicillin levels due to the repressive effect of glucose (139 mM) is about 13% when the sugar is added at inoculation time, whereas it increases to 59% when the medium is supplemented with 100 mM inorganic phosphate (56; F. Antequera and J. F. Martín, unpublished results). Inorganic phosphate (100 mM) has no effect per se on penicillin production under glucose-limited conditions or in lactose-based medium.

Interestingly, penicillin biosynthesis in *P. chrysogenum* is

also repressed by 2-deoxyglucose (73). This glucose analogue is phosphorylated to 2-deoxyglucose-6-phosphate but appears not to be metabolized further in glycolysis. This result suggests that signal transduction leading to glucose repression of penicillin biosynthesis genes proceeds by modification (perhaps phosphorylation) of a penicillin regulatory protein exerted by glucose or 2-deoxyglucose or a phosphorylated derivative of these sugars. The signal transduction cascade may involve a two-component regulatory system including a sensor protein and a partner response regulator protein. A glucokinase-deficient mutant (*glk-1*) of *P. chrysogenum* is simultaneously derepressed in carbon catabolite regulation of β -galactosidase and isopenicillin N synthase (5), suggesting that a common step of the signal transduction cascade (probably a sensor kinase) is involved in carbon catabolite regulation of both sugar utilization and penicillin biosynthesis. Hexokinase II has been implicated in carbon catabolite regulation of sugar utilization in yeasts (24, 35), but its involvement in signal transduction in filamentous fungi is obscure (76).

TRANSCRIPTION OF THE *pcbAB*, *pcbC*, AND *penDE* GENES OF *P. CHRYSOGENUM* IS STRONGLY REPRESSED BY GLUCOSE

Recent results in our laboratory showed that glucose repressed transcription of the three penicillin biosynthesis genes *pcbAB*, *pcbC*, and *penDE* (Fig. 2) when added at inoculation time to cultures of *P. chrysogenum* AS-P-78, but it had little repressive effect when added at 12 h of incubation and no effect when added after 24 or 36 h (37). Most of the *pcbAB* and *pcbC* transcripts are accumulated in the initial 36 to 48 h of the culture, while the *penDE* transcript (expressed from a different promoter) is synthesized later. The time window for the glucose repression effect was longer (up to 36 h) when the cultures were inoculated directly with conidia (Antequera and Martín, unpublished).

These results are consistent with the hypothesis that glucose (when added early to the culture) triggers a signal transduction cascade that modifies a carbon regulatory protein that interacts with the *pcbAB*, *pcbC*, and *penDE* promoters (Fig. 3). Repres-

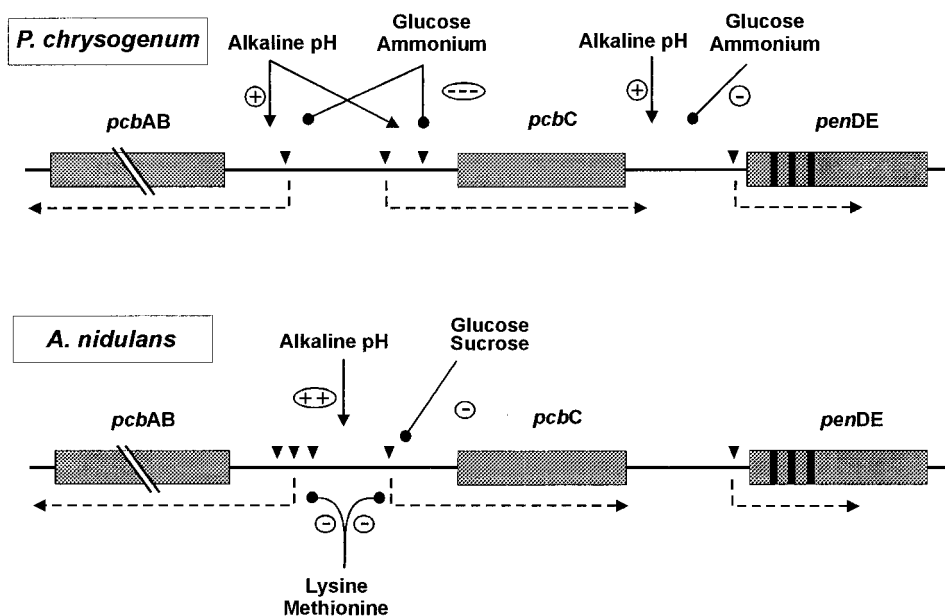


FIG. 2. Regulatory circuits controlling expression of the *pcbAB-pcbC* bidirectional promoter and the *penDE* promoter in *P. chrysogenum* and *A. nidulans*. Induction of gene expression is indicated by an arrow with \oplus , and repression of gene expression is indicated by \ominus with \bullet . The intensity of the induction or repression effect is shown by the number of + or - symbols. Transcription initiation sites (\blacktriangledown) are also shown.

sion by glucose of the three penicillin biosynthesis genes was also observed using the *lacZ* reporter gene coupled to each of the three promoters in single-copy transformants with the constructions integrated at the *pyrG* locus (37). Although the drastic decrease in the steady-state levels of the *pcbAB*, *pcbC*, and *penDE* transcripts might also be due to an increased degradation of the transcripts in glucose-supplemented cells, an effect of glucose on preventing transcription from the penicillin promoters is supported by the severe reduction in reporter β -galactosidase activity observed in single-copy constructions in which the *lacZ* gene was coupled to each of the three promoters (37). Similarly, repression of *pcbAB* and *penDE* was also established by the use of the *uidA* reporter system (28).

The residual expression of the *pcbC* and *penDE* genes in glucose-grown cultures was slightly increased at pH 6.8, 7.4, and 8.0, but alkaline pH values did not override the strong repression exerted by glucose. Transcription of the actin gene used as a control was not significantly affected by glucose or alkaline pH (37).

IS CARBON CATABOLITE REGULATION OF PENICILLIN BIOSYNTHESIS MEDIATED BY CreA?

All available evidence suggests that carbon catabolite regulation of penicillin biosynthesis is exerted by a carbon regulatory protein that transduces the nutritional signal (high or low glucose levels) to the penicillin gene promoters (Fig. 3). This carbon regulatory protein appears to be formed (or modified) in a short time window following inoculation of the penicillin fermentation, since the repressive effect is clearly lower when glucose is added after 12 to 24 h of incubation.

In *A. nidulans*, glucose repression of structural genes required for utilization of other alternative carbon sources is mediated by a transcriptional regulatory protein encoded by the *creA* gene (2–4, 22, 50). A similar *creA* gene in *Aspergillus niger* has also been reported (23), and there have been other

recent reports of similar genes in *Trichoderma resei* and *Trichoderma harzianum* (42), *Sclerotinia sclerotiorum* (92), and *Metharhizium anisopliae* (78). The CreA protein of filamentous fungi contains two Cys₂-His₂ zinc fingers which are similar to those of the yeast repressor MIG1, although the overall amino acid identity of CreA and MIG1 is not very high (67).

Two other proteins SSN6 (CYC8) and TUP1 are required for repression by MIG1 (89). Gene repression by glucose is relieved via a mechanism that requires the SNF1 protein kinase complex (84). When glucose is absent, the repressing activity of MIG1 is inhibited by protein kinase SNF1, which is activated upon removal of glucose. The MIG1 protein is readily phosphorylated in vitro by the SNF1 kinase under these conditions (69, 90). These results indicate that the MIG1 protein may be regulated by phosphorylation; these results are in good agreement with the observed involvement of phosphate in glucose repression of penicillin biosynthesis and the possible modification of a response regulator (Fig. 3).

The CreA and MIG1 proteins are closely related to a family of GC box binding proteins that includes the Wilms tumor and SP1 proteins (67). The CreA and MIG1 transcriptional repressors mediate carbon catabolite repression in *A. nidulans* and *Saccharomyces cerevisiae* by interaction of the two Cys₂-His₂ zinc fingers of the protein with the consensus 5'-SYGGGG-3' nucleotide sequence (S = C or G; Y = C or T; R = A or G) as shown by DNase I protection analysis (50, 66). Cubero and Scazzochio (19) expanded the consensus recognition sequence to 5'-SYGGRG-3'. However, in all *A. nidulans creA* mutants tested, glucose still represses the *ipnA* (= *pcbC*) mRNA (11, 26). Deletion of a 29-bp region which is protected by the CreA protein did not alter sucrose repression in *A. nidulans* (27). Mutations in *creB* and *creC* also have very minor effect on carbon regulation of penicillin biosynthesis (25). These results suggest that in *A. nidulans* a second *creA*-independent mechanism of carbon repression is involved in penicillin biosynthesis.

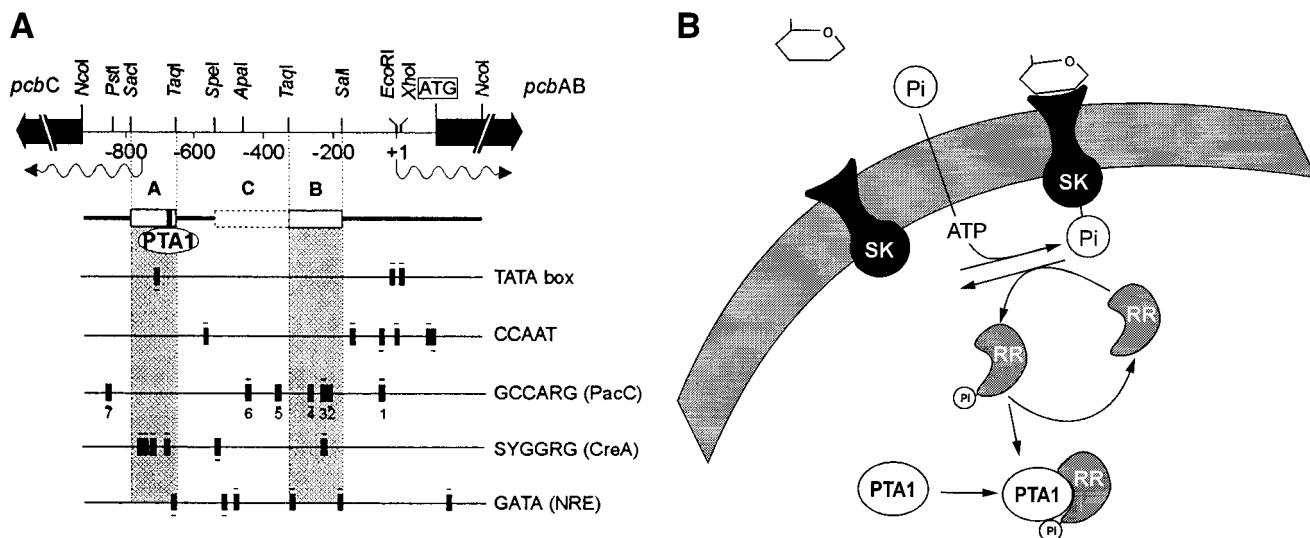


FIG. 3. (A) Scheme showing the locations of the TATA and CCAAT boxes and the PacC, CreA, and NRE consensus motifs in the *P. chrysogenum* *pcbAB-pcbC* bidirectional promoter. The regions corresponding to box A and box B are shaded. The PTA1 protein is shown bound to box A (see text). The transcripts of *pcbAB* and *pcbC* are indicated by wavy lines under the map at the top of the figure. (B) Model showing glucose signal transduction in *P. chrysogenum*. When glucose is absent, interaction of PTA1 with the modified (phosphorylated?) response regulator is proposed to activate penicillin gene expression. SK, sensor kinase; RR, response regulator; PTA1, penicillin transcriptional activator 1 (see text).

DIFFERENCES IN CARBON CATABOLITE REGULATION AND pH CONTROL OF PENICILLIN BIOSYNTHESIS IN *P. CHRYSOGENUM* AND *A. NIDULANS*

Sucrose (or glucose) repression of the *ipnA* (= *pcbC*) gene of *A. nidulans* is known (10, 26, 72), but Litzka and coworkers reported that expression of the *A. nidulans* *pcbAB* gene using reporter gene fusions was not repressed by the carbon source (53). The basic regulatory mechanisms of carbon and nitrogen regulation were initially believed to be similar in both fungi (28, 47). However, Suárez and Peñalva (86) proposed that the mechanisms of control by pH and carbon catabolite regulation are different in *P. chrysogenum* and *A. nidulans*. Our results support that conclusion (37).

The extracellular pH affects penicillin production in *A. nidulans* (79) and to a much lower extent in *P. chrysogenum* (18, 37). Espeso and coworkers (27) showed that external alkaline pH in *A. nidulans* overrides sucrose regulation of *ipnA*. By contrast, in *P. chrysogenum*, alkaline extracellular pH produces a small stimulation of transcription of *pcbAB*, *pcbC*, and *penDE*, but it does not override the strong catabolite regulation exerted by glucose (37). Brakhage (8, 9) has proposed that carbon source regulation of the *ipnA* gene in *A. nidulans* is mediated by a control mechanism independent of pH regulation. There are differences in the fine control of penicillin gene expression in *P. chrysogenum* and *A. nidulans* (86) that might be related to the higher expression of the penicillin genes in *P. chrysogenum* than in *A. nidulans*. The high expression of these genes in *P. chrysogenum* is due to the presence of a transcription-activating protein that binds to a specific sequence in the *pcbAB-pcbC* intergenic region (48).

PROTEIN BINDING SITES IN THE UPSTREAM REGION OF THE PENICILLIN BIOSYNTHESIS GENES

Several proteins in crude extracts of *P. chrysogenum* are able to bind to the *pcbAB-pcbC* intergenic region. Chu et al. (17) showed that proteins in partially purified extracts recognize the

TGCCAAG sequence. In parallel, Feng et al. (29) reported a nuclear factor (NF-A) that recognizes a slightly different binding sequence, GCCAAGCC. Both of these sites contain the CAAG motif and most likely correspond to the PacC binding sites (Fig. 3) that have been identified in *A. nidulans* [consensus sequence GCCA(A/G)G] (86, 88). Indeed, this motif is also located in the promoter region of the pH-regulated acid phosphatase *phoA* gene of *P. chrysogenum* (40).

The level of expression of the *P. chrysogenum* *pcbAB* gene was monitored by measuring the β -galactosidase activity of strains carrying sequential deletion derivatives of the *pcbAB* promoter fused to the *Escherichia coli* *lacZ* gene (Martín et al., Abstr. 8th Int. Symp. Genet. Ind. Microorg.). These fused constructions were targeted to the chromosomal *pyrG* locus of *P. chrysogenum* (37). Transformants integrated in single copy at the *pyrG* locus were selected. Analysis of the regulatory effect of deleting different DNA fragments of the *pcbAB-pcbC* intergenic region revealed two important promoter regions (boxes A and B) for expression of the *pcbAB* gene (Fig. 3). Using protein extracts from mycelia grown under carbon catabolite-repressing or -derepressing conditions, we have found DNA-binding proteins that specifically shift both promoter fragments. These proteins form DNA-protein complexes for each box: AG1 complex with box A in glucose-grown cells and AL1 in lactose-grown cells. Similarly, box B forms two complexes, BG1 and BG2, in glucose-grown cells and BL1 in lactose-grown cells (48).

Uracil interference assays showed that a protein in *P. chrysogenum* cell extracts interacts with the thymines in a palindromic heptanucleotide $\overline{\text{TTAGTAA}}$. The effects of point mutations and deletion of the entire $\overline{\text{TTAGTAA}}$ sequence supported the involvement of this sequence in the binding of a transcriptional activator that was named PTA1. The results of in vivo studies using constructions carrying point mutations in the TTAGTAA sequence or a deletion of the complete heptanucleotide confirmed that this intact sequence is required for protein binding and high-level expression of the *pcbAB* gene (48). The TTAGTAA sequence resembles the target sequence of BAS2

(PHO2), a factor required for expression of several genes in yeasts.

The *pcbAB-pcbC* intergenic region binding proteins were purified by heparin agarose chromatography, ammonium sulfate precipitation, gel filtration chromatography, and ion-exchange chromatography (Martín et al., Abstr. 8th Int. Symp. Genet. Ind. Microorg.). Formation of the DNA-PTA1 complex using extracts of glucose-grown *P. chrysogenum* cells is prevented by extracts of lactose-grown (glucose-limited) cells; i.e., a protein present in glucose-limited cells interacts with PTA1 or modifies this regulator, preventing formation of the AG1 DNA-repressor protein complex that occurs in glucose-grown cells (K. Kosalková and J. F. Martín, unpublished results) (Fig. 3). In summary, catabolite regulation of penicillin biosynthesis is mediated by protein-protein and protein-DNA interactions.

THE CCAAT BOX-BINDING PROTEIN COMPLEX PENR1 OF *A. NIDULANS*

Based on the results of a moving-window analysis of the *pcbAB-pcbC* intergenic region of *A. nidulans* and supported by band shift and methyl interference assays, a CCAAT-containing DNA motif that is recognized by a protein complex designated PENR1 was located 409 bp upstream of the *A. nidulans* *pcbAB* gene ATG initiation codon (72, 87). A 4-bp deletion within this motif led to an eightfold increase of *pcbAB* expression and to a simultaneous reduction of *ipnA* expression to about 30% of the original level (87). Another CCAAT box that is also recognized by the PENR1-protein complex is located 250 bp upstream of the transcriptional start site of *penDE*. Substitution of the CCAAT sequence by GATCC resulted in a fourfold reduction of expression of *penDE* (53). Similar CCAAT boxes are present in the promoters of many eukaryotic genes (43, 65).

A CCAAT-binding complex (the HAP complex) has been characterized in *S. cerevisiae*. It consists of at least four subunits, HAP2, HAP3, and HAP5 (forming a heterotrimeric complex essential for DNA binding) and HAP4, an acidic protein acting as a transcriptional activator. An *A. nidulans* gene designated *hapC*, similar to *S. cerevisiae* HAP3, was reported recently (70). In an *A. nidulans* strain in which *hapC* had been deleted, binding of the transcriptional factor AnCF (44) to the CCAAT boxes of the *amdS* and *gatA* genes did not occur, and indeed, Δ *hapC* strains grow very poorly on acetamide as the sole nitrogen source. These results indicate that the HapC protein is a functional part of the AnCF complex that binds to the CCAAY motif (45).

Further studies by Brakhage and coworkers indicated that the binding consensus sequence of the PENR1 complex is RR CCAAT(C/A)RCR (reviewed in reference 9), which matches the CCAAT site in the *amdS* promoter recognized by AnCF. Therefore, it is likely that PENR1 and AnCF share at least some protein components (85). Immunoprecipitation and supershift experiments with anti-HapC antibodies provided evidence that HapC is a component of the PENR1 complex (52).

Deletion or mutagenesis of the PENR1 binding sites increased eightfold the expression of the *pcbAB* gene, while expression of *pcbC* was reduced (87). In summary, CCAAT box I mediates a negative effect on *pcbAB* and a positive effect on *pcbC*. Similarly, mutation of CCAAT box II upstream of the *penDE* gene reduced expression of this gene (53). There are other CCAAT boxes in the *A. nidulans* *pcbAB-pcbC* bidirectional promoter, but they do not appear to bind PENR1 (53).

The real contribution of PENR1 to the control of penicillin gene expression is unclear. The lack of PENR1 in the PENR1 deletion mutant should lead to higher *pcbAB* transcript levels

and to a large increase in penicillin production, since expression of the *pcbAB* gene in *A. nidulans* is known to be limiting for penicillin biosynthesis (46). However, expression of a *pcbAB-uidA* gene fusion was not significantly affected in a Δ *hapC* *A. nidulans* mutant (nonfunctional PENR1) and the penicillin titers were reduced by about 30% in this mutant (52). Brakhage (9) proposed that in addition to PENR1, a repressor protein binds closely to or overlaps with the PENR1 binding site (CCAAT); this hypothesis would explain why the lack of PENR1 in the Δ *hapC* mutant did not result in increased *pcbAB* expression. Indeed, binding of proteins to sites adjacent to CCAAT sequences has been reported in *A. nidulans* (91) and in animal cells.

In summary, it seems that PENR1 represents a HAP-like protein which shares the HapC core protein with AnCF. However, both complexes may differ in ancillary proteins what would explain the different regulation of the penicillin biosynthesis and amidase formation.

AreA MEDIATES NITROGEN REGULATION OF PENICILLIN BIOSYNTHESIS

For many years, it has been known that ammonium ions decrease penicillin biosynthesis in *P. chrysogenum* (55, 77, 80). Ammonium concentrations above 40 mM repressed expression of the *uidA* reporter gene when fused to the *pcbAB* and *pcbC* promoters in *P. chrysogenum* (28).

A general mechanism of nitrogen regulation occurs in filamentous fungi and other microorganisms to ensure the proper utilization of different nitrogen sources. Ammonium ions or glutamine are favored and thus repress the permeases and enzymes required for the utilization of alternative nitrogen sources (nitrate, proteins, other amino acids, etc.). Nitrogen regulation is mediated in *N. crassa* by the *nit-2* gene product (34) and in *A. nidulans* by the *areA* gene product (49). Both genes encode positive regulatory factors with a single Cys-X₂-Cys-X₁₇-Cys-X₂-Cys zinc finger.

The *areA* gene of *P. chrysogenum* (named *nre*) was cloned by PCR and complementation of a *nit-2* mutant of *N. crassa* (38). These transcriptional factors recognize a consensus GATA sequence (61). Sometimes interaction occurs with two GATA elements separated by a varying number of base pairs (16). The Nit-2 protein of *N. crassa* and AreA of *A. nidulans* and *P. chrysogenum* have 98% homology in the GATA-recognizing domains, although the rest of the protein shows only about 30% overall identity.

There are six GATA sequences in the *P. chrysogenum* bidirectional *pcbAB-pcbC* promoter (Fig. 3). Interaction of the zinc finger fragment of the Nre protein of *P. chrysogenum* occurs specifically with a site containing two GATA sequences separated by 27 bp (38, 39). In separate experiments, the same GATA boxes were recognized by the *N. crassa* Nit-2 protein (28).

Although clear in vivo studies correlating alterations in GATA sequences or modifications of the Nre protein with changes in nitrogen regulation of penicillin biosynthesis have still not been done, it is likely that nitrogen regulation of penicillin biosynthesis in *P. chrysogenum* is mediated by the same regulatory protein involved in the nitrogen regulatory circuit.

Very little is known about the molecular mechanism of nitrogen regulation of penicillin biosynthesis in *A. nidulans*. When the bidirectional *pcbAB-pcbC* promoter region of *P. chrysogenum* was introduced into *A. nidulans*, it was sensitive to nitrogen regulation, indicating that the *A. nidulans* Nre protein acts on the *P. chrysogenum* GATA boxes (47). However, there are not tandem GATA boxes in the *A. nidulans* intergenic

pcbAB-pcbC promoter (there is a single GATA sequence), making it more unlikely that it might be recognized by Nre and perhaps explaining the lack of evidence of nitrogen regulation of penicillin biosynthesis in *A. nidulans*.

It is also worth mentioning that the intergenic *pcbAB-pcbC* region of the cephalosporin producer *Acremonium chrysogenum* contains 15 GATA sequences (64). Cephalosporin biosynthesis is known to be regulated by nitrogen, but no studies have been performed on the role of Nre in this fungus.

MUTATIONS AFFECTING EXPRESSION OF THE PENICILLIN BIOSYNTHESIS GENES IN TRANS

There are probably several domain-wide or pathway-specific regulatory genes affecting the cascade of penicillin gene expression. These have been hard to identify. Based on a search of clones expressing low levels of a chromogenic reporter gene fused to the *A. nidulans pcbC* promoter integrated at a specific locus, three mutations *prgA*, *prgB* (penicillin regulation), and *npeE1* (nonproducer of penicillin) were found (12, 71). These *prg* mutants exhibited reduced levels of penicillin biosynthesis. However, cloning of the corresponding genes complementing the *prgA*, *prgB*, and *npeE* mutations is required to support the involvement of these genes in penicillin biosynthesis.

During a screening of mutants blocked in penicillin biosynthesis, we isolated 10 mutants impaired in penicillin biosynthesis, two of which (*npe-2* and *npe-3*) showed reduced levels of the penicillin intermediate α -aminoadipyl-cysteinyl-valine and low activities of all the penicillin biosynthesis enzymes (14). It was proposed that these strains correspond to mutants with alterations in penicillin regulatory genes (*prg*). These strains provide a useful tool for further characterization of the penicillin regulatory mechanisms.

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REFERENCES

- Aharonowitz, Y., G. Cohen, and J. F. Martín. 1992. Penicillin and cephalosporin biosynthetic genes: structure, organization, regulation and evolution. *Annu. Rev. Microbiol.* **46**:461–496.
- Arst, H. N., Jr., and D. W. MacDonald. 1975. A gene cluster in *Aspergillus nidulans* with an internally located cis-acting regulatory region. *Nature* **254**:26–31.
- Arst, H. N., Jr., D. Tollervey, C. E. A. Dowzer, and J. M. Kelly. 1990. An inversion truncating the *creA* gene of *Aspergillus nidulans* results in carbon catabolite derepression. *Mol. Microbiol.* **4**:851–854.
- Bailey, C., and H. N. Arst, Jr. 1975. Carbon catabolite repression in *Aspergillus nidulans*. *Eur. J. Biochem.* **51**:573–577.
- Barredo, J. L., E. Alvarez, J. M. Cantoral, B. Díez, and J. F. Martín. 1988. A glucokinase-deficient mutant of *Penicillium chrysogenum* is derepressed in glucose catabolite regulation of both β -galactosidase and penicillin biosynthesis. *Antimicrob. Agents Chemother.* **32**:1061–1067.
- Barredo, J. L., J. M. Cantoral, E. Alvarez, B. Díez, and J. F. Martín. 1989. Cloning, sequence analysis and transcriptional study of the isopenicillin N synthase of *Penicillium chrysogenum* AS-P-78. *Mol. Gen. Genet.* **216**:91–98.
- Barredo, J. L., P. van Solingen, B. Díez, E. Alvarez, J. M. Cantoral, A. Kattavildar, E. B. Smaal, M. A. M. Groenen, A. E. Veenstra, and J. F. Martín. 1989. Cloning and characterization of acyl-CoA:6-APA acyltransferase gene of *Penicillium chrysogenum*. *Gene* **83**:291–300.
- Brakhage, A. A. 1998. Molecular regulation of penicillin biosynthesis in *Aspergillus (Emericella) nidulans*. *FEMS Microbiol. Lett.* **148**:1–10.
- Brakhage, A. A. 1998. Molecular regulation of β -lactam biosynthesis in filamentous fungi. *Microbiol. Mol. Biol. Rev.* **62**:547–585.
- Brakhage, A. A., P. Browne, and G. Turner. 1992. Regulation of *Aspergillus nidulans* penicillin biosynthesis and penicillin biosynthesis genes *acvA* and *ipnA* by glucose. *J. Bacteriol.* **174**:3789–3799.
- Brakhage, A. A., and G. Turner. 1995. Biotechnical genetics of antibiotic biosynthesis, p. 263–285. In U. Kück (ed.), *The Mycota II. Genetics and biotechnology*. Springer-Verlag KG, Berlin, Germany.
- Brakhage, A. A., and J. van den Bralle. 1995. Use of reporter genes to identify recessive *trans*-acting mutations specifically involved in the regulation of *Aspergillus nidulans* penicillin biosynthesis genes. *J. Bacteriol.* **177**:2781–2788.
- Caddick, M. X., A. G. Brownlee, and H. N. Arst, Jr. 1986. Regulation of gene expression by pH of the growth medium in *Aspergillus nidulans*. *Mol. Gen. Genet.* **203**:346–353.
- Cantoral, J. M., S. Gutiérrez, F. Fierro, S. Gil-Espinosa, H. van Liempt, and J. F. Martín. 1993. Biochemical characterization and molecular genetics of nine mutants of *Penicillium chrysogenum* impaired in penicillin biosynthesis. *J. Biol. Chem.* **268**:737–744.
- Chater, K., and M. Bibb. 1997. Regulation of bacterial antibiotic production, p. 59–105. In H. Kleinkauf and H. von Döhren (ed.), *Biotechnology: products of secondary metabolism*. VCH Verlag, Weinheim, Germany.
- Chiang, T. Y., and G. A. Marzluf. 1994. DNA recognition by the NIT2 nitrogen regulatory protein: importance of the number, spacing, and orientation of GATA core elements and their flanking sequences upon NIT2 binding. *Biochemistry* **33**:576–582.
- Chu, Y.-W., D. Renno, and G. Saunders. 1995. Detection of a protein which binds specifically to the upstream region of the *pcbAB* gene in *Penicillium chrysogenum*. *Curr. Genet.* **27**:184–189.
- Chu, Y.-W., D. Renno, and G. Saunders. 1997. Extracellular pH affects regulation of the *pcbAB* gene in *Penicillium chrysogenum*. *Appl. Microbiol. Biotechnol.* **47**:250–254.
- Cubero, B., and C. Scazzocchio. 1994. Two different, adjacent and divergent zinc finger binding sites are necessary for CRE-mediated carbon catabolite repression in the proline gene cluster of *Aspergillus nidulans*. *EMBO J.* **13**:407–415.
- Cundliffe, E. 1989. How antibiotic-producing organisms avoid suicide. *Annu. Rev. Microbiol.* **43**:207–233.
- Díez, B., S. Gutiérrez, J. L. Barredo, P. van Solingen, L. H. M. van de Voort, and J. F. Martín. 1990. The cluster of penicillin biosynthetic genes. Identification and characterization of the *pcbAB* gene encoding the α -aminoadipyl-cysteinyl-valine synthetase and linkage to the *pcbC* and *penDE* genes. *J. Biol. Chem.* **265**:16358–16365.
- Dowzer, C. E. A., and J. M. Kelly. 1991. Analysis of the *creA* gene, a regulator of carbon catabolite repression in *Aspergillus nidulans*. *Mol. Cell. Biol.* **11**:5701–5709.
- Drysdale, M. R., S. E. Kolze, and J. M. Kelly. 1993. The *Aspergillus niger* carbon catabolite repressor encoding gene, *creA*. *Gene* **130**:241–245.
- Entian, K.-D., and F. K. Zimmermann. 1982. New genes involved in carbon catabolite repression and depression in the yeast *Saccharomyces cerevisiae*. *J. Bacteriol.* **151**:1123–1128.
- Espeso, E. A., J. M. Fernández-Cañón, and M. A. Peñalva. 1995. Carbon regulation of penicillin biosynthesis in *Aspergillus nidulans*: a minor effect of mutations in *creB* and *creC*. *FEMS Microbiol. Lett.* **126**:63–68.
- Espeso, E. A., and M. A. Peñalva. 1992. Carbon catabolite repression can account for temporal pattern of expression of a penicillin biosynthetic gene in *Aspergillus nidulans*. *Mol. Microbiol.* **6**:1457–1465.
- Espeso, E. A., J. Tilburn, H. N. Arst, Jr., and M. A. Peñalva. 1993. pH regulation is a major determinant in expression of a fungal penicillin biosynthetic gene. *EMBO J.* **12**:3947–3956.
- Feng, B., E. Friedlin, and G. A. Marzluf. 1994. A reporter gene analysis of penicillin biosynthesis gene expression in *Penicillium chrysogenum* and its regulation by nitrogen and glucose catabolite repression. *Appl. Environ. Microbiol.* **60**:4432–4439.
- Feng, B., E. Friedlin, and G. A. Marzluf. 1995. Nuclear DNA-binding proteins which recognize the intergenic control region of penicillin biosynthetic genes. *Curr. Genet.* **27**:351–358.
- Fierro, F., J. L. Barredo, B. Díez, S. Gutiérrez, F. J. Fernández, and J. F. Martín. 1995. The penicillin gene cluster is amplified in tandem repeats linked by conserved hexanucleotide sequences. *Proc. Natl. Acad. Sci. USA* **92**:6200–6204.
- Fierro, F., S. Gutiérrez, B. Díez, and J. F. Martín. 1993. Resolution of four large chromosomes in penicillin-producing filamentous fungi: the penicillin gene cluster is located on chromosome II (9.6 Mb) in *Penicillium notatum* and chromosome I (10.4 Mb) in *Penicillium chrysogenum*. *Mol. Gen. Genet.* **241**:573–579.
- Fierro, F., and J. F. Martín. 1999. Molecular mechanism of chromosomal rearrangement in fungi. *Crit. Rev. Microbiol.* **25**:1–17.
- Fierro, F., E. Montenegro, S. Gutiérrez, and J. F. Martín. 1996. Mutants blocked in penicillin biosynthesis show a deletion of the entire penicillin gene cluster at a specific site within a conserved hexanucleotide sequence. *Appl. Microbiol. Biotechnol.* **43**:597–604.
- Fu, Y. H., and G. A. Marzluf. 1990. *nit-2*, the major positive-acting nitrogen regulatory gene of *Neurospora crassa*, encodes a sequence-specific DNA-binding protein. *Proc. Natl. Acad. Sci. USA* **87**:5331–5335.
- Gancedo, J. M. 1998. Yeast carbon catabolite repression. *Microbiol. Mol. Biol. Rev.* **62**:334–361.

36. Gutiérrez, S., F. Fierro, J. Casqueiro, and J. F. Martín. 1999. Gene organization and plasticity of the β -lactam genes in different filamentous fungi. *Antonie Leeuwenhoek* 75:81–94.
37. Gutiérrez, S., A. T. Marcos, J. Casqueiro, K. Kosalková, F. J. Fernández, J. Velasco, and J. F. Martín. 1999. Transcription of the *pcbAB*, *pcbC* and *penDE* genes of *Penicillium chrysogenum* AS-P-78 is repressed by glucose and the repression is not reversed by alkaline pHs. *Microbiology* 145:317–324.
38. Haas, H., B. Bauer, B. Redl, G. Stöffler, and G. A. Marzluf. 1995. Molecular cloning and analysis of *nre*, the major nitrogen regulatory gene of *Penicillium chrysogenum*. *Curr. Genet.* 27:150–158.
39. Haas, H., and G. A. Marzluf. 1995. NRE, the major nitrogen regulatory protein of *Penicillium chrysogenum* binds specifically to elements in the intergenic promoter regions of nitrate assimilation and penicillin biosynthetic gene clusters. *Curr. Genet.* 28:177–183.
40. Haas, H., B. Redl, E. Friedlin, and G. Stöffler. 1992. Isolation and analysis of the *Penicillium chrysogenum* *phoA* gene encoding a secreted phosphate-repressible acid phosphatase. *Gene* 113:129–133.
41. Hönliger, C., and C. P. Kubicek. 1989. Regulation of δ -(L- α -aminoacyl)-L-cysteine-D-valine and isopenicillin N biosynthesis in *Penicillium chrysogenum* by the α -aminoacyl-D-glutamate pool size. *FEMS Microbiol. Lett.* 65:71–76.
42. Ilmen, M., C. Thrane, and M. Penttilä. 1996. The glucose repressor gene *cre1* of *Trichoderma*: isolation and expression of a full-length and a truncated mutant form. *Mol. Gen. Genet.* 251:451–460.
43. Johnson, P. F., and S. L. McKnight. 1989. Eukaryotic transcriptional regulatory proteins. *Annu. Rev. Biochem.* 58:799–839.
44. Kato, M., A. Aoyama, F. Naruse, T. Kobayashi, and N. Tsukagoshi. 1997. An *Aspergillus nidulans* nuclear protein, AnCP, involved in enhancement of Taka-amylase A gene expression, binds to the CCAAT-containing *taaG2*, *amdS*, and *gatA* promoters. *Mol. Gen. Genet.* 254:119–126.
45. Kato, M., A. Aoyama, F. Naruse, Y. Tateyama, K. Hayashi, M. Miyazaki, P. Papagiannopoulos, M. A. Davis, M. J. Hynes, T. Kobayashi, and N. Tsukagoshi. 1998. The *Aspergillus nidulans* CCAAT-binding factor, AnCP/AnCF, is a heteromeric protein analogous to the HAP complex of *Saccharomyces cerevisiae*. *Mol. Gen. Genet.* 257:404–411.
46. Kennedy, J., and G. Turner. 1996. δ -(L- α -aminoacyl)-L-cysteine-D-valine synthetase is a rate limiting enzyme for penicillin production in *Aspergillus nidulans*. *Mol. Gen. Genet.* 253:189–197.
47. Kolar, M., K. Holzmann, G. Weber, E. Leitner, and H. Schwab. 1991. Molecular characterization and functional analysis in *Aspergillus nidulans* of the 5'-region of the *Penicillium chrysogenum* isopenicillin N synthetase gene. *J. Biotechnol.* 17:67–80.
48. Kosalková, K., A. T. Marcos, F. Fierro, V. Hernando-Rico, S. Gutiérrez, and J. F. Martín. 2000. A novel heptameric sequence (TTAGTAA) is the binding site for a protein required for high level expression of *pcbAB*, the first gene of the penicillin biosynthesis in *Penicillium chrysogenum*. *J. Biol. Chem.* 275:2423–2430.
49. Kudla, B., M. X. Caddick, T. Langdon, N. M. Martinez-Rossi, S. E. Bennett, S. Silbey, R. W. Davis, and H. N. Arst, Jr. 1990. The regulatory gene *areA* mediating nitrogen metabolite repression in *Aspergillus nidulans*. Mutations affecting specificity of gene activation alter a loop residue of a putative zinc finger. *EMBO J.* 9:1355–1365.
50. Kulmburg, P., M. Mathieu, C. Dowzer, J. Kelly, and B. Felenbok. 1993. Specific binding sites in the ethanol regulon for the CREA repressor mediating carbon catabolite repression in *Aspergillus nidulans*. *Mol. Microbiol.* 7:847–857.
51. Laich, F., F. Fierro, R. E. Cardoza, and J. F. Martín. 1999. Organization of the gene cluster for biosynthesis of penicillin in *Penicillium nalgioense* and antibiotic production in cured dry sausages. *Appl. Environ. Microbiol.* 65:1236–1240.
52. Litzka, O., P. Papagiannopoulos, M. A. Davis, M. J. Hynes, and A. A. Brakhage. 1998. The penicillin regulator PENR1 of *Aspergillus nidulans* is a HAP-like transcriptional complex. *Eur. J. Biochem.* 251:758–767.
53. Litzka, O., K. Then Bergh, and A. A. Brakhage. 1996. The *Aspergillus nidulans* penicillin biosynthesis gene *aat* (*penDE*) is controlled by a CCAAT containing DNA element. *Eur. J. Biochem.* 238:675–682.
54. Martín, J. F. 1998. New aspects of genes and enzymes for β -lactam antibiotic biosynthesis. *Appl. Microbiol. Biotechnol.* 50:1–15.
55. Martín, J. F., and Y. Aharonowitz. 1983. Regulation of biosynthesis of β -lactam antibiotics, p. 189–228. In A. L. Demain and N. A. Solomon (ed.), *Antibiotics containing the β -lactam structure*. Springer, New York, N.Y.
56. Martín, J. F., J. Casqueiro, K. Kosalková, A. T. Marcos, and S. Gutiérrez. 1999. Penicillin and cephalosporin biosynthesis: mechanism of carbon catabolite regulation of penicillin production. *Antonie Leeuwenhoek* 75:21–31.
57. Martín, J. F., and S. Gutiérrez. 1995. Genes for β -lactam antibiotic biosynthesis. *Antonie Leeuwenhoek* 67:181–200.
58. Martín, J. F., S. Gutiérrez, and A. L. Demain. 1997. β -Lactams, p. 91–127. In T. Anke (ed.), *Fungal biotechnology*. Chapman & Hall, Weinheim, Germany.
59. Martín, J. F., and P. Liras. 1989. Organization and expression of genes involved in the biosynthesis of antibiotics and other secondary metabolites. *Annu. Rev. Microbiol.* 43:173–206.
60. Martín, J. F., M. J. López-Nieto, J. M. Castro, J. Cortés, J. Romero, F. R. Ramos, J. M. Cantoral, E. Alvarez, M. G. Domínguez, J. L. Barredo, and P. Liras. 1986. Enzymes involved in β -lactam biosynthesis controlled by carbon and nitrogen regulation, p. 41–47. In H. Kleinkauf (ed.), *Regulation of secondary metabolite formation*. Verlag Chemie, Basel, Switzerland.
61. Marzluf, G. A. 1997. Genetic regulation of nitrogen metabolism in the fungi. *Microbiol. Mol. Biol. Rev.* 61:17–32.
62. McCabe, A. P., M. B. R. Riach, S. E. Unkles, and J. R. Kinghorn. 1990. The *Aspergillus nidulans* *npeA* locus consists of three contiguous genes required for penicillin biosynthesis. *EMBO J.* 9:279–287.
63. McCabe, A. P., H. van Liempt, H. Palissa, S. E. Unkles, M. B. R. Riach, E. Pfeifer, H. von Döhren, and J. R. Kinghorn. 1991. δ -(L- α -aminoacyl)-L-cysteine-D-valine synthetase from *Aspergillus nidulans*—molecular characterisation of the *acvA* gene encoding the first enzyme of the penicillin biosynthetic pathway. *J. Biol. Chem.* 266:12646–12654.
64. Menne, S., M. Walz, and U. Kück. 1994. Expression studies with the bidirectional *pcbAB-pcbC* promoter region from *Acremonium chrysogenum* using reporter gene fusions. *Appl. Microbiol. Biotechnol.* 42:57–66.
65. Mitchel, P. J., and R. Tjian. 1989. Transcriptional regulation in mammalian cells by sequence specific DNA binding proteins. *Science* 245:371–378.
66. Nehlin, J. O., M. Carlberg, and H. Ronne. 1991. Control of yeast GAL genes by MIG1 repressor: a transcriptional cascade in the glucose response. *EMBO J.* 10:3373–3377.
67. Nehlin, J. O., and H. Ronne. 1990. Yeast MIG1 repressor is related to the mammalian early growth response and Wilms' tumour finger proteins. *EMBO J.* 9:2891–2898.
68. Newbert, R. W., B. Barton, P. Greaves, J. Harper, and G. Turner. 1997. Analysis of a commercially improved *Penicillium chrysogenum* strain series: involvement of recombinogenic regions in amplification and deletion of the penicillin biosynthesis gene cluster. *J. Ind. Microbiol. Biotechnol.* 19:18–27.
69. Ostling, J., M. Carlberg, and H. Ronne. 1996. Functional domains in the Mig1 repressor. *Mol. Cell Biol.* 16:753–761.
70. Papagiannopoulos, P., A. Andrianopoulos, J. A. Sharp, M. A. Davis, and M. J. Hynes. 1996. The *hapC* gene of *Aspergillus nidulans* is involved in the expression of CCAAT-containing promoters. *Mol. Gen. Genet.* 251:412–421.
71. Pérez-Esteban, B., E. Gómez-Pardo, and M. A. Peñalva. 1995. A *lacZ* reporter fusion method for the genetic analysis of regulatory mutations in pathways of fungal secondary metabolism and its application to the *Aspergillus nidulans* penicillin pathway. *J. Bacteriol.* 177:6069–6076.
72. Pérez-Esteban, B., M. Orejas, E. Gómez-Pardo, and M. A. Peñalva. 1993. Molecular characterization of a fungal secondary metabolism promoter: transcription of the *Aspergillus nidulans* isopenicillin N synthetase gene is modulated by upstream negative elements. *Mol. Microbiol.* 9:881–895.
73. Revilla, G. 1983. Regulation of penicillin biosynthesis in *Penicillium chrysogenum*. Ph.D. thesis. University of Salamanca, Salamanca, Spain.
74. Revilla, G., M. J. López-Nieto, and J. F. Martín. 1984. Carbon catabolite repression of penicillin biosynthesis by *Penicillium chrysogenum*. *J. Antibiot.* 37:781–789.
75. Revilla, G., F. R. Ramos, M. J. López-Nieto, E. Alvarez, and J. F. Martín. 1986. Glucose represses formation of δ -(L- α -aminoacyl)-L-cysteine-D-valine and isopenicillin N synthase but not penicillin acyltransferase in *Penicillium chrysogenum*. *J. Bacteriol.* 168:947–952.
76. Ruijter, G. J., and J. Visser. 1997. Carbon repression in aspergilli. *FEMS Microbiol. Lett.* 151:103–114.
77. Sánchez, S., M. E. Flores, and A. L. Demain. 1988. Nitrogen regulation of penicillin and cephalosporin fermentation, p. 121–136. In S. Sanchez-Esquivel (ed.), *Nitrogen source control of microbial processes*. CRC Press, Inc., Boca Raton, Fla.
78. Screen, S., A. Bailey, K. Charnley, R. Cooper, and J. Clarkson. 1997. Carbon regulation of the cuticle-degrading enzyme PR1 from *Metarhizium anisopliae* may involve a trans-acting DNA-binding protein CRR1, a functional equivalent of the *Aspergillus nidulans* CREA protein. *Curr. Genet.* 31:511–518.
79. Shah, A. J., J. Tilburn, M. W. Adlard, and H. N. Arst, Jr. 1991. pH regulation of penicillin production in *Aspergillus nidulans*. *FEMS Microbiol. Lett.* 77:209–212.
80. Shen, Y.-Q., J. Heim, N. A. Solomon, S. Wolfe, and A. L. Demain. 1984. Repression of β -lactam production in *Cephalosporium acremonium* by nitrogen source. *J. Antibiot.* 37:503–511.
81. Smith, D. J., M. K. R. Burnham, J. H. Bull, J. E. Hodgson, J. M. Ward, P. Browne, J. Brown, B. Barton, A. J. Earl, and G. Turner. 1990. β -Lactam antibiotic biosynthetic genes have been conserved in clusters in prokaryotes and eukaryotes. *EMBO J.* 9:741–747.
82. Smith, D. J., M. K. R. Burnham, J. Edwards, A. J. Earl, and G. Turner. 1990. Cloning and heterologous expression of the penicillin biosynthetic gene cluster from *Penicillium chrysogenum*. *Bio/Technology* 8:39–41.
83. Smith, D. J., A. J. Earl, and G. Turner. 1990. The multifunctional peptide synthetase performing the first step of penicillin biosynthesis is a 421 073 dalton protein similar to *Bacillus brevis* peptide antibiotic synthetases. *EMBO J.* 9:2743–2750.
84. Smith, F. C., S. P. Davies, W. A. Wilson, D. Carling, and D. G. Hardie. 1999. The SNF1 kinase complex from *Saccharomyces cerevisiae* phosphorylates the

- transcriptional repressor protein Mig1p *in vitro* at four sites within or near regulatory domain 1. FEBS Lett. **453**:219–223.
85. Steidl, S., P. Papagiannopoulos, O. Litzka, A. Andrianopoulos, M. A. Davis, A. A. Brakhage, and M. J. Hynes. 1999. AnCT, the CCAAT binding complex of *Aspergillus nidulans*, contains products of the *hapB*, *hapC*, and *hapE* genes and is required for activation by the pathway-specific regulatory gene *amdR*. Mol. Cell. Biol. **19**:99–106.
 86. Suárez, T., and M. A. Peñalva. 1996. Characterisation of a *Penicillium chrysogenum* gene encoding a PacC transcription factor and its binding sites in the divergent *pcbAB-pcbC* promoter of the penicillin biosynthetic cluster. Mol. Microbiol. **20**:529–540.
 87. Then Berg, K., O. Litzka, and A. A. Brakhage. 1996. Identification of a major *cis*-acting DNA element controlling the bidirectionally transcribed penicillin biosynthesis genes *acvA* (*pcbAB*) and *ipnA* (*pcbC*) of *Aspergillus nidulans*. J. Bacteriol. **178**:3908–3916.
 88. Tilburn, J., S. Sarkar, D. A. Widdick, E. A. Espeso, M. Orejas, J. Mungroo, M. A. Peñalva, and H. N. Arst, Jr. 1995. The *Aspergillus* PacC zinc finger transcription factor mediates regulation of both acidic- and alkaline-expressed genes by ambient pH. EMBO J. **14**:779–790.
 89. Treitel, M. A., and M. Carlson. 1995. Repression by SSN6-TUP1 is directed by MIG1, a repressor/activator protein. Proc. Natl. Acad. Sci. USA **92**:3132–3136.
 90. Treitel, M. A., S. Kuchin, and M. Carlson. 1998. Snf1 protein kinase regulates phosphorylation of the Mig1 repressor in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **18**:6273–6280.
 91. Van Heeswick, R., and M. J. Hynes. 1991. The *amdR* product and a CCAAT-binding factor bind to adjacent, possibly overlapping DNA sequences in the promoter region of the *Aspergillus nidulans amdS* gene. Nucleic Acids Res. **19**:2655–2660.
 92. Vautard, G., P. Cotton, and M. Fèvre. 1999. The glucose repressor CRE1 from *Sclerotinia sclerotiorum* is functionally related to CREA from *Aspergillus nidulans* but not to the Mig proteins from *Saccharomyces cerevisiae*. FEBS Lett. **453**:54–58.