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NOTES

Opposite Patterns of Expression of Two *Aspergillus nidulans* Xylanase Genes with Respect to Ambient pH

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The *Aspergillus nidulans* xylanase genes *xlnA* and *xlnB* are subject to regulation by ambient pH via the zinc finger transcription factor PacC. In the presence of D-xylose, *xlnA* is expressed under conditions of alkaline ambient pH while *xlnB* is expressed at acidic ambient pH. These data have been confirmed for acidity- and alkalinity-mimicking *A. nidulans* mutants.

In nature, many microbes are exposed to large variations in ambient pH and thus require both an efficient pH homeostatic system and a regulatory mechanism which ensures that those molecules exposed to the environment (such as certain permeases, small metabolites, and extracellular enzymes) are only produced under appropriate pH conditions. The ascomycete *Aspergillus nidulans* is able to grow over a wide pH range (14). The regulatory circuit controlling pH regulation in this microorganism has been analyzed both genetically and molecularly. As an example, this system regulates the secretion of alkaline phosphatase in alkaline growth conditions and acid phosphatase in acidic environments (2).

pH regulation of gene expression in *A. nidulans* is mediated by the wide-domain zinc finger transcription factor PacC (16). In alkaline culture conditions, this factor is converted to its truncated functional form in response to the ambient pH signal transduced by the products of six genes (*palA*, *-B*, *-C*, *-F*, *-H*, and *-I*) and is able to activate the expression of those genes whose products are appropriate at alkaline ambient pH and repress those whose expression is suited to acidic pH (1, 3, 10, 11, 16). Activation is exercised by binding to the consensus target sequence 5′-GCCARG-3′ (5). We have previously shown that *A. nidulans* produces three xylanases when grown on D-xylose as the sole carbon source: one minor xylanase (X_{24}), encoded by *xlnB*, and two major xylanases (X_{22} and X_{34}), encoded by *xlnA* and *xlnC*, respectively (6, 7, 9, 12). In this article we demonstrate pH regulation of the expression of the *xlnA* and *xlnB* genes.

The nucleotide sequences of the upstream regions of the *xlnA* and *xlnB* genes have been determined and reveal the presence of two and one consensus PacC binding sites, respectively (Fig. 1). The occurrence of such binding targets suggests that the transcription of these genes might, at least in part, be regulated by this transcription factor.

Transcription of the xylanase genes was analyzed in mycelia grown under acidic (pH ~4.5), neutral (pH ~6.5), and alkaline (pH ~7.5) growth conditions. Wild-type conidia were inoculated in minimal medium (MM) (13) supplemented with 0.5% (wt/vol) Casamino Acids and 1% (wt/vol) D-fructose as the sole

xlnA promoter:

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-661 TCCTGGAAGTGCGTTGATCATTATTCCCCGAAAATGTAGTACCCAGTAAGTGGTCTAGCG
-601 GTGGCTATGGTAGGACATCTATGCCTAAGCTGGAGTTCTCATTGAACGTGTACCGGCCGA
-541 TTGCCCTAAACTCTGATTGAGAGCCGGAAACCTCATCTACCTGATGCTCAGGGGCCATCC
-481 AATAGCTTCCGATAGCATTACAGACAGATGGACTCGTCTTGGCCCACGGGTCTAGAACAG
-421 TCGCCGGAGCTGCCTCTATTTGAAACGGAGCTGAACCATGATACTTAAGCGTGCCAAGCG
-361 GCGCCGTTTCCCACTGGAACAAGGAGCAATAGAATTCTGCAGAGATTCTTCATTCAGGCT
-301 ATTCAGCAATTCGGTTTGTGGAGCGGATCGGGGTCCACTGGGTTTAGTCTGGGGTTTTTC
-241 TTTGCCCGCATGGGCTCTAGCACATGCACAGCTTGCAGTTGCTGCTACGCTATCTGGGAA
-181 AACGAATGGCTATTCAGGAGTTTATAACCAAAAGAGCCGGAAACAGGCTGATTGCCCTCT
-121 CACGGGGAGACGTTGTACTTCTGATCCAGAGGCTATTAACCGGACACTACCTATAAAGGA
 -61 GGTAGCATTCCTTTCTGTCCGGCTCCCAGATTCCAACAACCCAACTGACAGGATCAGCAC
  -1 AATG
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xlnB promoter:

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-640 CTTGTGAGTAGAAGCATCGTGCCAGACATCACACGCAATACAGCTTCCCTCAGCATTTTT
-590 TCGATACCCAAAACTCAGTTAACAACTTGCAGCCTAGAGTTGATCAGGACTTCTGCCGGA
-530 GATATCTCCATACGTACCGGAGGCCACTGTTCAGGCCAATTTTCCCGCAGAGAGATCGCT
-470 GATCTTCACCCGCAATTCTTCAAAGCTTCTACTCAGCCAATCAGATCGACGGCCCGTGCC
-410 TATTCTGTATTCTACCGGTGGTTCAGCCTATATTCTGCAGAGCATATTTGGATTGGCGGC
-350 ACGATATTGAAGCTTCTGGCAAAGCTTAAACGTATATGCCAAGACTGTCAATATACTTGA
-290 GACACAAACCCAAATTAGCCTATTCACGGTGCGAAACAAGACTTCTCACTTGACACCCGT
-230 CTATGTTTCATCCTGACACATGCGTGGATTCAAATTCGCCATCGAAAGAATGGTAGCCAT
-170 TCAAAGGCTTCGTGGATTAACCGGCAGCCTAAAGAATATTAACCCTCAAACACTCCGTCC
-110 CCACAGTCAGCAGTACCCCCAGGTATCATATGATGGACGTGACTATAAAGCAACGTCCCG
 -50 GCACGTCTCAACACGTCCCAGTCTGCCTCAACACTTCAACAACCGGCAACATG
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FIG. 1. DNA sequences of the *xlnA* and *xlnB* gene promoters (EMBL accession no. Z49892 and Z49893, respectively). The translational initiation codon is shown in bold, and consensus PacC binding sites are shown in bold and underlined.

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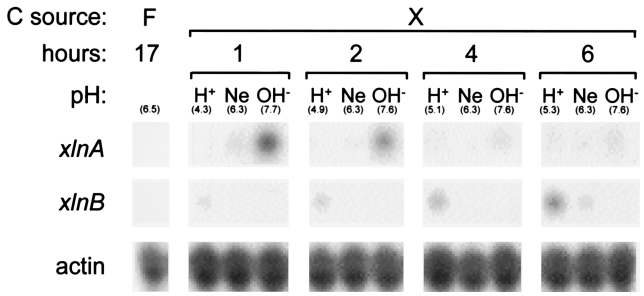

FIG. 2. Expression of *xlnA* and *xlnB* genes in *A. nidulans* wild-type mycelium in response to external pH. Carbon (C) sources present were D-fructose (F) and D-xylose (X). pH conditions are indicated as follows: H^+, acidic; Ne, neutral; and OH^-, alkaline. The actual pHs of the culture media at the time of recovery of mycelia for RNA isolation are indicated in parentheses. Actin gene expression is shown as a loading control.

carbon source. Incubation was done for 17 h at 37°C with orbital shaking, and the mycelia were subsequently transferred to buffered media containing 1% (wt/vol) D-xylose as the sole carbon source for 1, 2, 4, and 6 h. Total RNA was prepared and analyzed by Northern blotting. Figure 2 shows that the *xlnA* transcript appears shortly after transfer and only in alkaline growth conditions. In contrast, the *xlnB* transcript is detected after 4 h and mainly in acidic growth conditions. These data suggest pH regulation of the expression of *xlnA* and *xlnB*.

In order to investigate whether this response to ambient pH is mediated by PacC, we examined the transcript levels of the two genes in different pH mutant genetic backgrounds. Wild type, *pacC*^{c}14 (an extreme alkalinity-mimicking mutant) (2, 16), *pacC*$^{+/-}$20205 (an acidity-mimicking *pacC* mutant [17]), and *palA*1 (a mutant in the signal transduction pathway mimicking acidic conditions) (1, 2, 10) mycelia were grown in D-fructose MM as noted above and transferred to MM (pH 6.5) containing 1% (wt/vol) D-xylose as the sole carbon source. At different time points after transfer (1, 2, 4, and 6 h) total RNA was isolated and analyzed by Northern blotting. Figure 3 shows that the alkalinity-mimicking *pacC*^{c}14 mutation simultaneously results in elevated levels of the *xlnA* transcript and reduced levels of the *xlnB* messenger. In contrast, the acidity-mimicking *pacC*$^{+/-}$20205 and *palA*1 mutations yield the opposite pattern of expression. The highest level of expression of *xlnA* occurs in the *pacC*^{c}14 mutant background, whereas that of *xlnB* occurs in the *palA* background.

The analyses described above show an absolute correspondence between the patterns of transcription of the xylanase genes under different ambient pH conditions and those observed in the pH mutant backgrounds. Specific transcription of

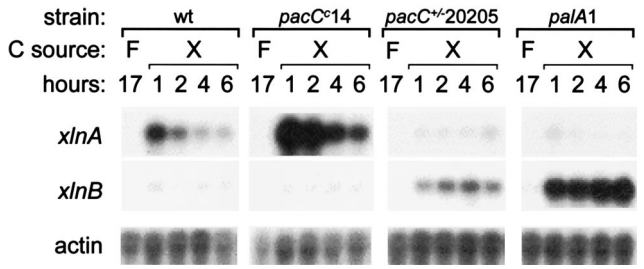

FIG. 3. *xlnA* and *xlnB* expression in *A. nidulans* pH-regulatory mutants. Carbon (C) sources present were D-fructose (F) and D-xylose (X). Measurements at the time of recovery of mycelia for RNA isolation showed that the pH of all culture fluids fell within the range of 5.9 to 6.5. Actin gene expression is shown as a loading control.

the *xlnA* and *xlnB* genes requires both the presence of an inducer (D-xylose) and optimal ambient pH. In *Aspergillus niger* the regulation by pH of the two major acidic extracellular proteases was investigated by Northern analysis (8). Due to the absence of pH-regulatory mutants of *A. niger*, this study was carried out by varying the pH of the culture medium. In the present study, pH regulation of the expression of two *A. nidulans* xylanase-encoding genes was investigated under different conditions of ambient pH and for different alkalinity- and acidity-mimicking mutants. This constitutes the first demonstration of the control of xylanase gene expression by ambient pH via the wide-domain pH regulator PacC. It is interesting to note the noncoincident, reversed patterns of expression of *xlnA* and *xlnB* with respect to pH and the time course of induction. The former might be related to the fact that *xlnB* codes for an acidic xylanase stable at acidic pH and *xlnA* encodes a neutral xylanase that is less stable at acidic pH (6, 7).

Including the data presented in this report, four *A. nidulans* alkaline-expressed genes, *ipnA*, *pacC*, *prtA*, and *xlnA*, have been studied with respect to pH expression (4, 16). In agreement with the proposed model for PacC as a transcriptional activator (11, 16), each of these genes contains several consensus PacC binding sites in the promoter. Only in the case of *ipnA* has the physiological relevance of the PacC sites been determined (5). The mechanism for the negative action of PacC in the regulation of acid-expressed genes is poorly understood. Interestingly, *pacA*, the only acid-expressed *A. nidulans* gene studied so far, contains an upstream PacC site, but it lies well within the transcribed region and quite close to the putative initiator codon (15, 15a). In contrast, the PacC site in *xlnB* lies much more nearly in the region where PacC sites occur in alkaline-expressed promoters.

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REFERENCES

1. **Arst, H. N., Jr., E. Bignell, and J. Tilburn.** 1994. Two new genes involved in signalling ambient pH in *Aspergillus nidulans*. Mol. Gen. Genet. **245:**787–790.
2. **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.
3. **Denison, S. H., M. Orejas, and H. N. Arst, Jr.** 1995. Signaling of ambient pH in *Aspergillus* involves a cysteine protease. J. Biol. Chem. **270:**28519–28522.
4. **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.
5. **Espeso, E. A., and M. A. Peñalva.** 1996. Three binding sites for the *Aspergillus nidulans* PacC zinc-finger transcription factor are necessary and sufficient for regulation by ambient pH of the isopenicillin N synthase gene promoter. J. Biol. Chem. **271:**28825–28830.
6. **Fernández-Espinar, M. T., F. Piñaga, P. Sanz, D. Ramón, and S. Vallés.** 1993. Construction of an *Aspergillus nidulans* multicopy transformant for the *xlnB* gene and its use to purify the minor X_{24} xylanase. FEMS Microbiol. Lett. **113:**223–228.
7. **Fernández-Espinar, M. T., F. Piñaga, L. H. de Graaff, J. Visser, D. Ramón, and S. Vallés.** 1994. Purification, characterisation and regulation of the synthesis of an *Aspergillus nidulans* acidic xylanase. Appl. Microbiol. Biotechnol. **42:**555–562.
8. **Jarai, G., and F. Buxton.** 1994. Nitrogen, carbon, and pH regulation of extracellular acidic proteases of *Aspergillus niger*. Curr. Genet. **26:**238–244.
9. **MacCabe, A. P., M. T. Fernández-Espinar, L. H. de Graaff, J. Visser, and D. Ramón.** 1996. Identification, cloning and isolation of the *Aspergillus nidulans* *xlnC* gene encoding the 34-kDa xylanase. Gene **175:**29–33.
10. **Negrete-Urtasun, S., S. H. Denison, and H. N. Arst, Jr.** 1997. Characterization of the pH signal transduction pathway gene *palA* of *Aspergillus nidulans* and identification of possible homologs. J. Bacteriol. **179:**1832–1835.

11. **Orejas, M., E. A. Espeso, J. Tilburn, S. Sarkar, H. N. Arst, Jr., and M. A. Peñalva.** 1995. Activation of the *Aspergillus* PacC transcription factor in response to alkaline ambient pH requires proteolysis of the carboxy-terminal moiety. Genes Dev. **9:**1622–1632.
12. **Pérez-González, J. A., L. H. De Graaff, J. Visser, and D. Ramón.** 1996. Molecular cloning and expression in *Saccharomyces cerevisiae* of two *Aspergillus nidulans* xylanase genes. Appl. Environ. Microbiol. **62:**2179–2182.
13. **Pontecorvo, G.** 1953. The genetics of *Aspergillus nidulans*. Adv. Genet. **5:**141–238.
14. **Rossi, A., and H. N. Arst, Jr.** 1990. Mutants of *Aspergillus nidulans* able to grow at extremely acidic pH acidify the medium less than wild type when grown at more moderate pH. FEMS Microbiol. Lett. **66:**51–54.
15. **Sarkar, S., M. X. Caddick, E. Bignell, J. Tilburn, and H. N. Arst, Jr.** 1996. Regulation of gene expression by ambient pH in *Aspergillus*: genes expressed at acid pH. Biochem. Soc. Trans. **24:**360–363.

15a. **Sarkar, S., E. A. Espeso, J. Tilburn, and H. N. Arst, Jr.** Personal communication.

16. **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 acid- and alkaline-expressed genes by ambient pH. EMBO J. **14:**779–790.
17. **Widdick, D. A., and H. N. Arst, Jr.** Unpublished data.