A cis-Acting Sequence Homologous to the Yeast Filamentation and Invasion Response Element Regulates Expression of a Pectinase Gene from the Bean Pathogen Colletotrichum lindemuthianum*

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Phytopathogenic fungi secrete hydrolytic enzymes that degrade plant cell walls, notably pectinases. The signaling pathway(s) that control pectinase gene expression are currently unknown in filamentous fungi. Recently, the green fluorescent protein coding sequence was used as a reporter gene to study the expression of CLPG2, a gene encoding an endopolygalacturonase of the bean pathogen Colletotrichum lindemuthianum. CLPG2 is transcriptionally induced by pectin in the axenic culture of the fungus and during formation of the appressorium, an infection structure specialized in plant tissue penetration. In the present study, promoter deletion and mutagenesis, as well as gel shift mobility assays, allowed for the first time identification of cis-acting elements that bind protein factors and are essential for the regulation of a pectinase gene. We found that two different adjacent DNA motifs are combined to form an active element that shows a strong sequence homology with the yeast filamentation and invasion response element. The same element is required for the transcriptional activation of CLPG2 by pectin and during appressorium development. This study strongly suggests that the control of virulence genes of fungal plant pathogens, such as pectinases, involves the formation of a complex of transcriptional activators similar to those regulating the invasive growth in yeast.

Saprophytic and phytopathogenic filamentous fungi secrete extracellular enzymes that degrade plant cell wall polymers. Among them, pectinases are the subject of intense research, because pectin degradation contributes to fungal pathogenicity in several host-pathogen systems (1–4) and is of considerable interest for various biotechnological processes. Pectinase gene expression is regulated at the transcriptional level by environmental conditions such as the pH of the medium (5, 6) and by carbon sources, being induced by pectin and pectic components (polygalacturonic acid, galacturonic acid, arabinose, and rhamnose) and repressed by glucose (6–8). Whereas the regulatory pathways that control pectinase gene expression are well documented in phytopathogenic bacteria (9), little is known about the regulation of fungal pectinases. Recently, ccSNF1, a gene encoding a protein homologous to the yeast protein kinase SNF1 required for expression of glucose-repressed genes, was isolated from the maize pathogen Cochliobolus carbonum (10). Mutants disrupted in this gene showed a reduced pathogenicity, and genes coding for hydrolytic enzymes were down-regulated.

Colletotrichum lindemuthianum is a pathogenic fungus that is the causal agent of bean anthracnose. Conidia germinate on the surface of the aerial part of the plant and differentiate a specialized cell called appressorium, which allows the parasite to penetrate plant tissues (11). During the first stages of infection, C. lindemuthianum establishes a biotrophic interaction with the host plant. 3–4 days post-inoculation, the parasite develops secondary hyphae and becomes necrotrophic, causing tissue necrosis. In a previous work, we characterized two endoPG genes, CLPG1 and CLPG2, from C. lindemuthianum (12, 13). CLPG1 encodes the major endoPG isoform that is produced during axenic culture of the fungus on pectin and during the necrotrophic stage of infection. CLPG2 is early and only transiently expressed at the onset of plant infection and on pectin. Recently we developed the use of GFP1 as a reporter gene to study the transcriptional regulation of CLPG2 (14). The promoter of CLPG2 allowed expression of the reporter gene during the germination of conidia on pectin medium and during appressorium formation both on a glass slide and during pathogenesis, which indicates that diverse signals can induce endoPG gene transcription. The main goal of the present study was to look for cis-acting elements in the promoter of CLPG2 involved in the induction of this gene under various situations. Deletions of the promoter delineated a 27-bp fragment required for CLPG2 induction on pectin and during appressorium formation. It is shown that this DNA fragment binds protein factors and contains two essential elements that are highly homologous to the yeast filamentation and invasion response element (FRE).

EXPERIMENTAL PROCEDURES

Fungal Culture and Protoplast Transformation—C. lindemuthianum race β was maintained on synthetic agar medium as described (15) or grown on 9-cm cellophane disks laid down onto the surface of 20 ml of solid ANM medium (2% malt extract, 0.1% bactopeptone, 2% D(+)-glucose, 2% agar) in Petri dishes. After inoculation with 10⁶ conidia/dish, the mycelium was allowed to develop for 48 h at 24 °C before being transferred for 10 h on solid medium supplemented with apple pectin (16) or for 24 h on bean cotyledon leaves in a highly humid atmo-

1 The abbreviations used are: GFP, green fluorescent protein; FRE, filamentation and invasion response element; PRE, pheromone response element; TCS, TEA/ATTS consensus sequence(s); PLE, PRE-like elements; TLE, TCS-like element; GMSA, gel mobility shift assay; MAPK, mitogen-activated protein kinase; endoPG, endopolygalacturonase.

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cis-Acting Elements Regulating Expression of a Fungal endoPG Gene

### Table I

| Name        | Sequence 5' to 3'                      | Gene and position |
|-------------|---------------------------------------|-------------------|
| pg2-490     | GGATCGGATCCATCATCAAATG3C               | PG2: -490 to -473 |
| pg2-90      | GGATCCCTCGACGAAGCTCAAGACATCTCC         | PG2: -90 to -60  |
| pg2-63      | GGATCCGAGCTGTCACAAAGGGTGTCC           | PG2: -63 to -40  |
| pg2-490A27  | CTCGCTGAGAATCCACGGAGATTGGAGGCACGCAG   | PG2: -117 to -33 |
| pg2+178     | GCGCACTGAGAGGAGAGGAGAATCAGAGCCATC     | PG2: complementary t of +201 to +178 |
| pg2+1207    | CGCTCTGGTTTGAACGCTCAAGTGGACAC         | PG2: complementary of +1236 to +1207 |
| pg2-90:TE   | CGCGATCCGAGAAGCTCAAGAAGAGAGAGGCAGG   | PG2: -90 to -56  |
| gpd-S       | GGATCCGCTGAGAAGCTCAAGAGGAGAGGAGG     | GPDA: -405 to -380 |
| gpd-R       | CAGCATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGAT
cis-Acting Elements Regulating Expression of a Fungal endoPG Gene

Fig. 1. Effect of promoter deletions on expression of CLPG2-GFP fusions. A, organization of the 668-bp promoter region indicating the two transcription start sites at positions +1 and +12 and the putative TATA box (gray square). Promoter fragments, corresponding to nucleotides −490 to +178 (PG2-490), −90 to +178 (PG2-90), −63 to +178 (PG2-63), and a full-length promoter in which 27 bp were removed from nucleotide −90 to nucleotide −63 (PG2-490Δ27) were fused to the GFP reporter gene. B, detection by fluorescence microscopy of GFP expression under the control of the various CLPG2 promoter fragments. For each construct and condition, the transformants were observed after 24 h of growth on glucose (column a) or pectin medium (column b) and after 48 h on glass slides for appressorium formation (column c) or on hypocotyl segments for pathogenesis induction (column d). Scale bar, 10 μm. A, appressorium; C, conidia; GT, germ tube.

RESULTS

Deletion Analysis of the CLPG2 Promoter—Previous experiments using transgenic strains containing a CLPG2-GFP construct showed that a promoter fragment of 668 bp was sufficient to induce expression of the reporter gene in the mycelium grown on pectin and during appressorium formation both in vitro on glass slides and in planta at early stages of pathogenesis (14). The general organization of the CLPG2 promoter (PG2-490) is depicted on Fig. 1A. Two transcription start sites were identified 178 and 186 bp upstream from the initiation codon by 5' rapid amplification of cDNA ends-PCR, corresponding to positions +1 and +12, respectively. A putative TATA (TATAA) box was located at position −40.

To identify cis-acting elements involved in transcriptional control in vitro and in planta, CLPG2-GFP constructs harboring sequential 5' deletions of the promoter region of CLPG2 were introduced in the genome of C. lindemuthianum (Fig. 1A). For each construct, at least four independent transformants were isolated. The presence of GFP was verified by PCR, and the number of GFP copies integrated into the fungal genome was evaluated by Southern blotting. Accumulation of GFP in conidia was detected by fluorescence microscopy. Fig. 1B shows that induction of GFP expression by pectin and appressorium formation was of the same magnitude in transformants containing the full-length promoter (PG2-490) or the promoter deleted to −90 (PG2-90). However, deletion of a further 27 bp (construct PG2-63) abolished induction of the reporter gene during the three growth conditions. Removal of this 27-bp sequence only from the full-length promoter (construct PG2-490Δ27) did not modify the promoter activity, showing that additional regulatory elements were present upstream on the promoter (Fig. 1B).

To confirm that the construct PG2-90 induced accumulation of GFP transcript on pectin medium, a Northern blot experiment was performed on RNA extracted from the mycelium grown on glucose and transferred on glucose or pectin. Hybridization with probes corresponding to CLPG2 DNA or to the GDP coding sequence showed the simultaneous accumulation of CLPG2 and GFP RNAs on pectin medium (data not shown). Taken together these results indicated that the 27-bp DNA fragment contained regulatory elements allowing GFP transcription in the fungus grown on pectin medium and during appressorium formation in vitro and in planta at the very first stages of pathogenesis.

Gel Mobility Shift Assays—The 27-bp fragment corresponding to nucleotides −90 to −63 was chosen as a probe for GMSA (Fig. 2). Protein extracts were prepared from the mycelium, which was grown on glucose and subsequently transferred either on glucose or pectin or on bean leaves. In the latter case, the fungus was allowed to grow on a cellophane sheet laid down onto the surface of cotyledonary leaves of the susceptible bean cultivar P125. Microscopy analysis showed that in these conditions, 24–48 h after inoculation, appressoria were fully differentiated and started to develop primary hyphae through the cellophane sheet, thereby mimicking the first stages of pathogenesis. Removal of the cellophane sheet allowed recovery of the mycelium.

In preliminary experiments, we compared the formation of protein-DNA complexes obtained with protein prepared from isolated nuclei or from mycelium. In each case, the same major protein-DNA complex was observed on the gel as shown in Fig. 2 (A and B) with proteins extracted from the mycelium. Accordingly, total protein extracts were used for further experiments. The signal was more intense when the probe was incubated with protein extracts prepared from the mycelium grown on pectin or during pathogenesis, but a signal was also observable with proteins extracted from the mycelium grown on glucose. Competition experiments carried out with an excess of the unlabeled DNA probe efficiently eliminated appearance of the signal, whereas nonspecific competitor DNA did not affect the binding (Fig. 2, A and B). The addition of deoxycholic acid, a chaotrophic agent that dissociates protein complexes, led to disappearance of the signal (Fig. 2C), suggesting that protein-protein interactions are required for the formation of the protein-DNA complex.

DNA Sequences Homologous to the Yeast Filamentation Response Element Are Involved in Protein Binding—Analysis of the 27-bp sequence revealed the presence of two different putative regulatory elements (Fig. 3A). The first element showed a strong homology with the eukaryotic TCS motif containing the TEA/ATTS consensus sequence CATTCC, which binds transcriptional factors belonging to the TEA/ATTS family. Therefore, this element was designated TLE because it contains the sequence GATTCC. A second class of element containing the consensus sequence WNAAT(1,2)A was called the PRE-like element (PLE) according to its homology with the yeast DNA regulatory sequence WGAAGCA called the pheromone response element (PRE), which interacts with the STE12 trans-acting factor. Four TLEs and PLEs were detected along the CLPG2 promoter (Fig. 3A). In yeast, a combination of TCS and PRE has been shown to play a major role in the regulation
of genes during the filamentation and invasive response through the binding of TEC1 and STE12 transcription factors (21). Remarkably, TLEs and PLEs are also arranged in tandem in the promoter of \textit{CLPG2}.

To determine whether the binding of protein factors on the 27-bp fragment was due to the presence of TLEs and PLEs, GMSA was performed with the 27-bp fragment as the probe and double-stranded oligonucleotides corresponding to three repetitions of either TLE-90 (3\times TLE-90), PLE-79 (3\times PLE-79), or PLE-71 (3\times PLE-71) as cold competitors (Fig. 3B). A 50 molar excess of 3\times PLE-79 and 3\times PLE-71 was sufficient to compete very efficiently with the formation of the complex (Fig. 3B), whereas a 1000-fold excess of 3\times TLE-90 was necessary to disrupt the binding (data not shown). Thus, the proteins binding to the 27-bp DNA fragment appeared to have more affinity for a combination of TLE and PLE than for three TLEs. To further identify mutations in PLEs that alter the binding of nuclear proteins, a competition experiment was performed in the presence of different double-stranded mutated PLE-79 oligo-
gonucleotides containing one or two base substitutions compared with the wild type sequence (Fig. 3C). Oligonucleotides M1, M2, and M4, which contained PLE mutated in the first nucleotide, the central adenine doublet, and the terminal adenine residue, respectively, failed to inhibit the formation of the complex. Thus, the nucleotides residues that are totally conserved between the yeast STE12 binding site and the C. linde\textsuperscript{muthianum} PLEs are essential for the binding of protein factors.

In yeast, TEC1 and STE12 bind as heterodimers on FRE. To determine whether a combination of PLE and TLE can also bind protein factors, GMSA was performed with double-strand DNA fragments corresponding to the 27-bp fragment mutagenized in each of the elements (Fig. 4A). The mutations consisted of replacing A doublets by G doublets. For both experiments, quantification of the signal corresponding to the DNA-protein complex was done using a PhosphorImager system. C, effect of double mutations in TLE-90 and PLE-79 (lane 2) present in the PG2-90 construct (lane 1) on the induction of GFP accumulation by pectin. The data represent the means ± S.D. of the specific fluorescence measured on protein extracts from four independents transformants grown on glucose or pectin.

**Fig. 4.** A combination of TLE and PLE is required to bind protein factors. A, GMSA was performed with protein extracts from pectin-induced mycelium and 10 fmol of radiolabeled probe corresponding to the wild type 27-bp region (lane 1) or the 27 bp mutated in TLE, PLE, or both (lanes 2-7). The mutations consisted of replacing A doublets by G doublets. B, GMSA was performed with protein extracts from pectin induced mycelium and 10 fmol of radiolabeled probe corresponding to the wild type 27-bp region in the absence (−) or in the presence of a 50-fold molar excess of unlabeled competitors. The mutations consisted of replacing A doublets by G doublets. For both experiments, quantification of the signal corresponding to the DNA-protein complex was done using a PhosphorImager system. C, effect of double mutations in TLE-90 and PLE-79 (lane 2) present in the PG2-90 construct (lane 1) on the induction of GFP accumulation by pectin. The data represent the means ± S.D. of the specific fluorescence measured on protein extracts from four independents transformants grown on glucose or pectin.

**Fig. 5.** Effect of three copies of PLE on GFP expression. A, a synthetic promoter was constructed by fusing three repetitions of PLE-71 to a 405-bp constitutive promoter from the A. nidulans GPDA gene. A, comparison of fluorescence intensity of transformants expressing GFP under the control of PG2-490 (column 1), GPDA (column 2), and 3×PLE-GPDA (column 3). The data were obtained by quantifying at least 110 fluorescence microscopic views from three independent transformants grown on glucose or pectin. B, microscopic views of representative transformants grown on glucose or pectin. Scale bar, 10 μm. C, conidia; GT, germ tube.

**Fig. 6.** Model for the transcriptional regulation of CLPG2. A, under repressive conditions (glucose medium), binding of factors related to the yeast STE12 (gray ovals) represses expression of CLPG2. B, upon induction by pectin and pathogenesis (appressorium differentiation), the activation of a MAPK pathway leads to the binding of a transcription factor related to the yeast TEC1 (black symbols) and to a formation of an heterodimer with a STE12-like protein that induces transcription of CLPG2.
duced the competition, showing that these mutations decreased the affinity for nuclear factors. Altogether, these experiments showed that a combination of TLE-90 and PLE-71 binds nuclear factors with higher efficiency than two PLEs.

In a further experiment, it was determined that transformants harboring GFP under the control of a CLPG2 promoter mutated in the TLE-90 and PLE-71 expressed only a basal level of fluorescence (Fig. 4C). Thus, the ability of DNA fragments to form a complex with proteins in vitro is correlated with the promoter activity in vivo.

Transcriptional Repression Mediated by PRE-like Elements—In yeast, KSS1 is a negative regulator of invasive growth and binds in its unphosphorylated form to STE12, thereby repressing the transcription of target genes containing FRE (22). Because mutation of one PLE increased the formation of the protein-DNA complex observed in inducing conditions, we tested the hypothesis that PLEs could be involved in the repression of CLPG2 in the fungus grown on glucose. A synthetic promoter was constructed by fusing three copies of PLE-71 to a constitutive promoter from the A. nidulans GPDA gene (23). This DNA fragment was fused to GFP, and C. lindemuthianum strains harboring this construct were analyzed by quantitative fluorescence microscopy. The level of fluorescence exhibited by the strains expressing the GFP gene under the control of the GPDA promoter was of the same magnitude when grown on glucose or pectin. However, the addition of 3×PLE-71 strongly reduced the expression on glucose, whereas accumulation of GFP was unchanged on pectin medium (Fig. 5). Thus, a combination of PLEs is sufficient to confer glucose repression on a constitutive promoter.

DISCUSSION

Despite the long lasting interest of plant pathology and biotechnology in fungal pectinases, the molecular mechanisms governing their expression are still unknown. In the present study, we report on the identification of regulatory elements involved in the transcriptional control of CLPG2, a pectinase gene of C. lindemuthianum. These elements bind protein factors and are essential for expression of a reporter gene during saprophytic growth of the fungus on pectin and during interaction with the host plant.

A search for homology with previously described nuclear factor-binding sites revealed the presence of putative TCS. TCS have been reported to bind transcription factors of the TEA/ATTS family mainly involved in developmental processes in fungi and higher eukaryotes (24). A second sequence was identified by homology with the yeast PRE found in promoters of genes involved in the mating response (25). A combination of the CLPG2 elements, called TLE and PLE, was sufficient to bind protein factors and ensure promoter activity. These effects were totally lost upon mutagenesis of these elements. Moreover, a construct comprising three PLEs fused to a constitutive promoter was able to repress the constitutive expression of a reporter gene in the fungus grown on glucose medium. Taken together, these results show that the regulation of CLPG2 requires the binding of transcription factors to a DNA sequence comprising TLE and PLE. Up to now, such combinations of elements were not reported in true filamentous fungi.

In yeast, a combination of TCS and PRE, also called the FRE, mediates the binding of an heterodimer formed by the association of the transcriptional activators TEC1 and STE12 (21, 26). The MAPK KSS1 plays a key role in the transcriptional control of genes regulated by FRE both by derepression and activation. Indeed, the unphosphorylated form of KSS1 is part of a protein complex that also contains STE12, TEC1, and the inhibitory proteins DIG1 or DIG2. Upon phosphorylation through a MAPK cascade, KSS1 dissociates from the complex, thereby destabilizing the STE12-DIG association leading to derepression of the target genes. Simultaneously, phosphorylation of STE12 by KSS1 activates the STE12-driven transcription. The transcriptional repression activity mediated by C. lindemuthianum PLEs strongly suggests that similar mechanisms operate in this fungus.

The hypothesis that CLPG2 is regulated by transcription factors related to the yeast STE12 and TEC1 proteins is strengthened by the recent finding that expression of a polypeptide, galacturonase, POU1, is induced in yeast during the haploid-invasive growth and diploid pseudohyphal development (27). This induction requires a functional filamentation MAPK pathway, including the presence of TEC1 and STE12. Thus, during the yeast invasive response, the same molecular mechanisms activate genes involved in cell elongation and differentiation and genes that encode extracellular proteins, allowing the successful colonization of their natural substrates. Related results have been obtained for two animal pathogens, Candida albicans and Cryptococcus neoformans. In C. albicans, the TEA/ATTS transcription factor CaTEC1 controls hyphal development and expression of genes encoding extracellular proteases (28). Similarly, a STE12 homologue from C. neoformans regulates expression of virulence genes, notably encoding an extracellular phospholipase (29). Recently, a STE12-like gene that plays an essential role in sexual reproduction, STEA, was isolated from a true filamentous fungus, A. nidulans (30). However, the role of this factor in the expression of pectinase genes was not investigated.

The likely involvement of transcriptional activators homologous to STE12 in the regulation of the C. lindemuthianum pectinase gene CLPG2 could be related to the presence of MAPKs belonging to the FUS3/KSS1 family. Interestingly, MAPKs homologous to the yeast FUS3/KSS1 have been identified in a number of phytopathogenic fungi (31) where they play essential roles in pathogenicity. Thus, disruption of the MAPK FMK1 in the tomato root pathogen Fusarium oxysporum greatly reduced the expression of the endopeptidase lyase gene pl1 (32).

According to the results presented in this paper, homologues of the yeast STE12 and TEC1 factors are likely to play a key role in pectinase gene expression, suggesting that mechanisms regulating invasive growth share striking similarities between saprophytic and pathogenic microorganisms. Such similarities were already pointed out for dimorphic fungi able to switch between a yeast and a multicellular invasive filamentous form (31).

From our data and the above report, it emerges that the regulation of CLPG2 might comply with the model proposed in Fig. 6. Thus, in repressive medium (glucose medium), the MAPK pathway would not be activated, and binding of protein factors related to the yeast STE12 would repress expression of the gene. In inductive medium (pectin medium) or at early stages of pathogenesis (appressorium development), activation of the MAPK pathway would induce a rearrangement of the protein-DNA complex comprising a STE12-like factor and a protein belonging to TEA/ATTS family of transcription factors. Isolation and functional analysis of these transcription factors are currently underway. Their characterization will help unravel the signaling pathways leading to induction of fungal pathogenicity.

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2 C. Herbert and B. Dumas, unpublished observations.
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