Core Promoter Structure in the Oomycete Phytophthora infestans

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We have investigated the core promoter structure of the oomycete Phytophthora infestans. The transcriptional start sites (TSS) of three previously characterized P. infestans genes, Piexo1, Piexo3, and Piendo1, were determined by primer extension analyses. The TSS regions were homologous to a previously identified 16-nucleotide (nt) core sequence that overlaps the TSS in most oomycete genes. The core promoter regions of Piexo1 and Piendo1 were investigated by using a transient protoplast expression assay and the reporter gene β-glucuronidase. Mutational analyses of the promoters of Piexo1 and Piendo1 showed that there is a putative core promoter element encompassing the TSS (−2 to +5) that has high sequence and functional homology to a known core promoter element present in other eukaryotes, the initiator element (Inr). Downstream and flanking the Inr is a highly conserved oomycete promoter region (+7 to +15), hereafter referred to as FPR (flanking promoter region), which is also important for promoter function. The importance of the 19-nt core promoter region (Inr and FPR) in Piexo1 and Piendo1 was further investigated through electrophoretic mobility shift assays (EMSA). The EMSA studies showed that (i) both core promoters were able to specifically bind a protein or protein complex in a P. infestans whole-cell protein extract and (ii) the same mutations that reduced binding of the EMSA complex also reduced β-glucuronidase (GUS) levels in transient expression assays. The consistency of results obtained using two different assays (GUS transient assays [in vivo] and EMSA studies [in vitro]) supports a convergence of inference about the relative importance of specific nucleotides within the 19-nt core promoter region.

We are interested in the oomycetes because they are a group of eukaryotes that include important pathogens of plants, animals, and insects in addition to saprophytes (1). Although oomycetes have a fungus-like growth, both phylogenic and phenotypic analyses have shown that they are not fungi but belong to the kingdom Chromista (8, 49).

Very little is known about transcription initiation of protein-coding genes in the kingdom Chromista, which includes a group of diverse organisms such as brown algae and diatoms. In oomycetes only one core promoter element has been characterized; it is in the pip1 gene of Phytophthora infestans. This promoter contains a 17-bp CT-rich element that is important for transcription. It has an Inr-like element (CTTCCTTCT) at the 5′ region, 24 bp upstream from the TSS. However, the functional significance of this putative Inr-like element, not present in any other known oomycete gene, is unclear (9). In contrast, a highly conserved 16-nucleotide (nt) putative core promoter element is present at the TSSs of almost all known oomycete genes (40). Other than sequence conservation, the functional significance of this element is unknown and has not been investigated. Oomycete core promoter regions are further characterized by the absence of canonical TATA elements (18).

In this study we have investigated the core promoter structure of two putative β-1,3-glucanase genes, Piexo1 and Piendo1, from the oomycete P. infestans. The expression of Piexo1 is regulated during development, being highly expressed in mycelia and sporangia, but not in zoospores and germinating cysts. Piendo1 is also differentially regulated, being expressed in mycelia, sporangia, and germinating cysts but not in zoospores (32). We have studied the promoter regions (sequence upstream from the start ATG) of these two genes by using a transient protoplast expression system. Sites that were de-

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ected as important in the transient assays were further investigated by electrophoretic mobility shift assays.

MATERIALS AND METHODS

Primer extension analyses. The TSSs of three previously characterized P. infestans genes, Pexo1, Pexo3 and Pendo1 (32), were determined by primer extension analyses. P. infestans RNA was isolated from mycelia grown for 10 days in pea broth (14) by using a hot phenol method (37). The extracted RNA was DNase treated according to the manufacturer's instructions (Invitrogen Corporation, Carlsbad, Calif.). Primer Ext-exo1 (TCCATGGAGTAGGATGCGGCG) was used for primer extension analysis of Pexo1, primer Ext-exo2 (AAGCTCTA TGGAGCTTCAGGGCAGC) was used for Pexo3, and primer Ext-endo1 (CTGGCCGATTTGTGAAGTGTGATC) was used for Pendo1. Primers (1 pmol each) were end labeled and annealed to 60 μg of total RNA in 1× first strand buffer (Invitrogen) at 85°C for 30 min. The mixture was heated for 5 min at 65°C prior to annealing. After primer annealing, Superscript II reverse transcriptase (400 U) (Invitrogen Corporation), deoxyxynucleoside triphosphates (20 mM each), RNAseOut (Invitrogen) (80 U), and dithiothreitol (DTT) (800 mM) were added in a total volume of 40 μl. Primer extension was done at 42°C for 1 h. The reaction mixture was ethanol precipitated and loaded onto a 4% nondenaturing polyacrylamide gel. Sequencing ladders of the three genes were obtained by using the fmol DNA cycle sequencing system according to the manufacturer's instructions (Promega). Primer extension analyses were repeated twice using independent RNA extractions.

Comparison of oocyte core promoter regions. A literature search revealed that the TSS has been reported for only 15 oocyte genes (in species of Phytophthora, Bremia, and Achlya). The sequences surrounding the TSSs of all these genes were compared by alignment. The previously identified conserved 16 nt core sequence that encompasses the transcriptional start site of oocyte genes was used as a reference point (40). We also searched for the 16 nt core sequence in the upstream regions of 15 oocyte genes (Phytophthora, Bremia, and Achlya) for which the TSSs have not been determined.

Construction of reporter plasmids. Plasmids containing Pexo1 promoter–beta-glucuronidase(GUS) (pEXO) and Pendo1 promoter–GUS (pENDO) transcriptional fusions were constructed using plasmid pHAM35G, kindly provided by H. S. Judelson (University of California, Riverside). pHAM35G is a pUC19-based vector which includes the 920-bp promoter of Bremia lactucae fused to the bacterial GUS gene (uidA) and the 550-bp ham34 terminator of B. lactucae (21). The ham34 promoter is flanked by HindIII and NcoI restriction sites in pHAM35G. The vectors pEXO and pENDO were constructed by excising the ham34 promoter from pHAM35G with the promoters (regions upstream from the translational start codon) of Pexo1 and Pendo1, using the HindIII and NcoI restriction sites (restriction sites of the promoter region of pHAM34 were conservatively bracketed). The PCR product was ampliﬁed using primers PP-EX1 (GCATGAAGCTTAATGACCACATCTTTAAA) and PP-EX2 (GTATGCCATGGTCTGCCTACAGATTCGAA). The promoter region (1 kb) of Pendo1 was ampliﬁed using primer PP-EN1 (GCATGAAGCTTAATGACCACATCTTTAAA) and primer PP-EN2 (ATACGGCATTGTCCTGATGACATTGCGAA). The introduced HindIII restriction sites in PP-EX1 and PP-EN1 and NcoI restriction sites in PP-EX2 and PP-EN2 are underlined. All PCRs were done as previously described (32). The promoter regions were ampliﬁed using Platinum TaqDNA Polymerase High Fidelity (Invitrogen Corporation) and an annealing temperature of 65°C. The structures of the constructs were veriﬁed by sequencing the 3′ promoter and 5′ GUS gene junction, as well as by restriction digestion with HindIII and NcoI.

Transformation procedure for obtaining stable P. infestans transformants. Transformants of P. infestans (strain SA960008) expressing pEXO or pENDO were obtained as previously described (20, 21). Cotransformation of 107 protoplasts was done as previously described (20, 21). An equimolar amount (13 pmol) of each construct was tested in four independent experiments using 1 ml of protoplasts (107 protoplasts) per construct. Within each experiment two replicates of the relevant wild-type construct and two replicates of the promoter-GUS fusion constructs were used to ensure that DNA uptake between different tubes within an experiment, consisting of 8 to 10 tubes containing 1 ml of protoplasts, were equal. Since the two replicate wild-type treatments within an experiment differed only by 10 to 15%, we were conﬁdent that (i) DNA quantiﬁcation and isolation was not affecting GUS quantiﬁcation and (ii) within an experiment DNA uptake was comparable among all 10 to 15 transformation tubes. The transformed protoplasts were regenerated for 22 h and harvested as described previously (20). Harvested protoplasts (1 ml) were resuspended in 200 μl of GUS extraction buffer (12) and frozen in liquid nitrogen. The thawed protoplast suspensions were lysed by sonication for 1 min in a sonicator bath followed by 1 min on ice. The sonication was repeated three more times, and the last sonication for 2 min by using a sonicator bath set at a power of 60% of 12,000. Protein concentrations of the supernatant were determined by using the Bradford reagent (Bio-Rad). For each construct 100 μl of supernatant was incubated with 100 μl of 2 mM 4-methylumbelliferone (4-MU) at 37°C. The amount of product (4-methylumbelliferone [4-MU]) was measured after 1 h, using a fluorometer as described previously (12). The amounts of MU produced by the deletion and mutation plasmids were corrected for protein concentration and expressed as percentages relative to the amounts of MU produced by the corresponding wild-type plasmids (pEXO and pENDO) within each experiment.

Plasmid DNA for the transient expression assays was isolated using the Wizard Plus Maxiprep kit according to the manufacturer’s instructions (Promega). All constructs that gave relative GUS values of less than 50% relative to the wild-type plasmid were tested with two independent DNA plasmid preparations. This was done to ensure that reduced GUS levels seen in these mutated constructs were not due to bad plasmid quality or errors in DNA quantitation. Transient expression assays using these independent DNA plasmid preparations gave similar relative GUS levels when compared to the wild-type construct in each of the four to six independent experiments.

Whole-cell P. infestans protein extraction. A whole-cell protein extract from P. infestans mycelia was prepared using a slightly modiﬁed method of Green et al. (16). P. infestans was grown for 7 days in pea broth. Harvested mycelia were rinsed with distilled water and then washed twice in cold phosphate-buffered saline. Approximately 12 g (wet weight) of mycelia was blended in 100 ml of cold buffer A (40 mM Tris-HCl [pH 7.5], 5 mM MgCl2, 0.5 M sucrose, 10 mM β-mercaptoethanol, 0.8 M guanidinium thiocyanate, and 0.5 M diethylpyrocarbonate). The homogenized mycelia were filtered through Miracloth (Calbiochem, La-
NaCl was added to the filtrate to a final concentration of 0.5 M. The filtrate mixture was gently agitated for 30 min at 4°C and then spun at 40,000 rpm in a Beckman L7-65 centrifuge (50.2 Ti rotor) for 1 h. Proteins in the supernatant were precipitated by adding 0.3 g of ammonium sulfate/ml and stirring at 4°C for 30 min. One-tenth milliliter of 1 M NaOH was added for each 10 g of ammonium sulfate added. The precipitated proteins were collected by centrifugation at 11,000 rpm for 25 min in a Beckman rotor. The pellets were combined in 2.5 ml of buffer B (20 mM HEPES-KOH [pH 7.3], 40 mM KCl, 1 mM EDTA, 10% glycerol, 0.5 mM DTT, 0.8 mM PMSF, 10 μM leupeptin). The buffer was exchanged with buffer B, using a PD-10 desalting column according to manufacturer's instructions (Pharmacia Biotech, Piscataway, N.J.).

**RESULTS**

**Identification of the TSSs of *P. infestans*, *Piexo3*, and *Piendo1***

Primer extension analyses showed that the TSSs of *P. infestans*, *Piexo3*, and *Piendo1* mapped to a conserved 16-nt core sequence, present at the TSSs of most oomycete genes (40). The primer extension product for all three genes consisted of only one band, which mapped to an adenosine residue within the 16-nt core (Fig. 1). The region upstream from *P. infestans*, *Piexo3*, and 15% glycerol, 12 mM HEPES-KOH [pH 7.3], 0.8 mM PMSF, and 10 μM leupeptin. The competition assays were performed with double-strand non-labeled wild-type and mutant oligonucleotides at 200 and 400 μM molar excess concentrations for *Piexo1* and at 100 μM molar excess concentrations for *Piendo1*. Binding was done on ice for 10 min using non-labeled oligonucleotides, followed by addition of [γ-32P]ATP-labeled wild-type oligonucleotides and another 20-min incubation on ice. The samples were loaded onto a 4.5% Tris-borate-EDTA nondenaturing polyacrylamide gel, followed by electrophoresis at 300 V for 20 min at room temperature. Subsequently, the gels were dried and bands were visualized by PhosphorImager analyses (Molecular Dynamics Imaging System Storm 850). The band intensities were quantified by using the Molecular Dynamics Imagequant version 5 software.
and Piendo1 did not contain a conserved TATA element at approximately –30 from the TSS.

**TSSs of Piexo1 and Piendo1 reporter plasmids in stable P. infestans transformants.** Four *P. infestans* transformants expressing chimeric Piexo1 promoter-GUS (pEXO) and Piendo1 promoter-GUS (pENDO) reporter plasmids were obtained. The TSSs of these transformants, expressing the GUS gene driven by the promoter of either Piexo1 or Piendo1, mapped to the same adenosine residue that is used as the TSS in the native gene promoters (data not shown). This shows that transcriptional fusion of the promoters to the GUS gene, which introduced two cytosine residues at the 3′ region of the promoter, did not alter the TSS in the promoter. This also indicates that cryptic sites in the vector were not used for transcription initiation.

**Comparison of oomycete core promoter regions.** The sequences surrounding the TSSs of Piexo1, Piexo3, Piendo1 and other published oomycete genes were compared to the previously identified 16-nt core consensus sequence (40). The TSS regions of Piexo1, Piexo3, and Piendo1 all have high homology to the conserved 16-nt core sequence (Fig. 1). However, sequence comparison of the genes and published oomycete genes with known TSSs showed that the 16-nt core consensus sequence (GCTCAATTCATTTT) extends at the 3′ region up to position +15, forming a highly conserved 19-nt core sequence (Fig. 1). Within the 19-nt core consensus sequence (GCAANNNNNNTTTNG) the first adenine nucleotide is designated as +1 (Fig. 1). Investigation of the region upstream from the translational start codon in 18 oomycete genes (Phytophthora, Achlya, and Saproleagia) for which the TSS has not been determined supported the presence of this highly conserved 19-nt core sequence (Fig. 1).

Further investigation of the 19-nt core and putative 19-nt core sequences of oomycete genes, including Piexo1, Piendo1, and Piexo3, showed that there is a sequence very similar to that of the Inr present in metazoan and Trichomonas promoter regions (Fig. 1). The putative oomycete Inr has a suggested consensus sequence of YCAANNTTTT. The putative Inr overlaps the known TSSs of the genes, and in most of the genes the initiation of transcription is at the adenine residue within the putative Inr, indicated by +1 (Fig. 1).

**Scanning mutational analyses of the promoter of Piexo1.** The importance of the 16-nt core sequence present at –4 to +12 in Piexo1 was determined by first deleting this region from pEXO (Fig. 2, pEXO-delbox). Deletion of the 16-nt core sequence resulted in a severe reduction of promoter activity relative to the full-length wild-type promoter (Fig. 2, pEXO-delbox).

The importance of nucleotides in the –8 to +18 region of Piexo1 was determined by using scanning mutational analyses that introduced several two to four consecutive base pair mutations into the region (Fig. 2). Scanning mutational analyses revealed that bp –1 to +3 (pEXO*–1/+3) and bp +9 to +11 (pEXO*+9/+11) were very important for promoter function (Fig. 2). Furthermore, scanning mutational analyses suggested that the region directly downstream from the +9 to +11 region, at positions +13 to +15, is also important for promoter function (Fig. 2, pEXO*+13/+16 and pEXO*+14/+15).

**Point mutational analyses in the putative Inr of Piexo1 and Piendo1.** Scanning mutational analyses and sequence comparison showed that the region –1 to +5 contained a putative Inr that was important for promoter function (Fig. 1 and 2). To determine if nucleotides within the putative Inr were important for promoter function, point mutations were introduced into this region in pEXO and pENDO. The point mutational analyses showed that in both promoters the nucleotides at positions –1, +1, and +3 were most critical for promoter function (Fig. 3a and b).

**Mutational analyses of the region directly downstream from a putative Inr in Piexo1 and Piendo1.** Scanning mutational analyses suggested that the region directly downstream from the putative Inr in Piexo1 is also important for promoter function (Fig. 2). Therefore, we further investigated the effect of point mutations in this region in the context of the full-length Piexo1 promoter. Point mutations were introduced at positions +9 to +12 in pEXO1 (Fig. 3a). Point mutations at positions +9, +10, and +11 resulted in a reduction of promoter activity to less than 50% of that of the wild type (Fig. 3a, pEXO*+9, pEXO*+10, and pEXO*+11).

To determine if the importance of the region directly downstream of a putative Inr is unique to Piexo1, we also investigated the importance of this region in Piendo1. Similar to Piexo1, mutations at bases +9 to +11 in the promoter of Piendo1 also resulted in a reduction of promoter activity (Fig. 2, pEXO*+9/+11; Fig. 3b, pENDO*+9/+11). A mutation at +6 to +8 in Piendo1, which did not cause a reduction in promoter activity of Piexo1, did reduce (to ca. 50%) the activity of the promoter of Piendo1 (Fig. 2, pEXO*+6/+8, and Fig. 3b, pENDO*+6/+8).

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**FIG. 2.** Scanning mutational analyses of the *P. infestans* promoter Piexo1. The histogram shows the GUS activities of *P. infestans* protoplasts transformed with different mutated constructs of the full-length promoter of Piexo1. The sequence of the region in which mutations were introduced is shown to the left of the histogram. Mutations are indicated by lowercase letters, and the transcriptional start site is designated as A+1. There were no deletions in the promoters except where indicated by dashed lines. The activity of each mutated construct is shown as a percentage of the activity of the wild-type promoter (pEXO) and is the mean ± standard deviation of four independent experiments.
Deletion analyses of the promoter of Piexo1. Deletions at the 5′ region of the 993-bp Piexo1 promoter resulted in various reductions in promoter activity (Fig. 4). The TSS in the Piexo1 promoter is designated as +1, resulting in the untranslated region ending at +69 and the 5′ upstream promoter region being designated as −924. The shortest fragment of the 5′ deletion plasmids that still had promoter activity was a 75-bp promoter fragment containing bp −6 to +69 [Fig. 4, pEXO(−6 to +69)].

![Graph showing GUS activities of P. infestans protoplasts transformed with constructs pEXO and pENDO containing point mutations within the full-length promoters of Piexo1 and Piendo1.](image)

FIG. 3. Point mutational analyses of the putative Inr and the region directly downstream from the Inr in two P. infestans promoters, Piexo1 and Piendo1. Histograms show the GUS activities of P. infestans protoplasts transformed with constructs pEXO (a) and pENDO (b) containing point mutations within the full-length promoters of Piexo1 and Piendo1. The sequence of the region into which mutations were introduced is shown to the left of the histograms. Mutations are shown in bold and lowercase, and the transcriptional start site is indicated by +1. The activity of each mutated construct is shown as a percentage of the activity of the wild-type promoter (pEXO and pENDO) and is the mean ± standard deviation of four independent experiments.

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![Graph showing GUS activities of P. infestans protoplasts transformed with Piexo1-GUS constructs with deletions in the 5′ and 3′ promoter regions of Piexo1.](image)

FIG. 4. Deletion analyses of the Piexo1 promoter of P. infestans. The histogram shows GUS activities of P. infestans protoplasts transformed with Piexo1-GUS constructs with deletions in the 5′ and 3′ promoter regions of Piexo1. The activity of each deletion construct is shown as a percentage of the activity of the wild-type promoter (pEXO) and is the mean ± standard deviation of four independent experiments. The conserved 16-nt core sequence in the promoter of Piexo1 is indicated by a black square. The transcriptional start site (+1) is indicated by a right-angled arrow, and deletions are indicated by dashed lines.
Deletions at the 3’ promoter region of Piexo1 suggest that this region could be important for promoter function (Fig. 4). Only a low level of promoter activity was detected when bases +11 to +69 were deleted [Fig. 4, pEXO(−924 to +12)]. Interestingly, a construct that contained 10 bp more (+21 to +69 deleted) showed an increase in promoter activity [Fig. 4, pEXO(−924 to +22)]. These results suggest that bases +12 to +22 could be important for promoter function, although 3’ deletions can also affect mRNA’s stability and translation. Collectively, the 5’ and 3’ deletion analyses showed that only fragments containing the 19-nt core sequence were able to maintain some promoter activity and that the minimal promoter of Piexo1 could possibly consist of the region −6 to +12.

Gain-of-function constructs. A 75-bp region (−6 to +69) of Piexo1 [pEXO(−6 to +69)] containing the core promoter region was able to respond to a 493-bp upstream promoter fragment of Piexo1 (Fig. 4). The addition of the 493 upstream base pairs to the pEXO(−6 to +69) construct increased the promoter activity of pEXO(−6 to +69) from 36 to 76% [Fig. 4, pEXO(−924]+69) and pEXO(−6 to +69)].

P. infestans EMSAs. EMSAs were used to determine if promoter fragments of Piexo1 (31 bp) and Piendo1 (36 bp), each containing the 19-nt core sequence, had specific interactions with proteins in a P. infestans whole-cell protein extract (Fig. 5). In EMSA studies of both promoter regions, excess molar amounts (200 and 400× for Piexo1 and 50 and 100× for Piendo1) of nonlabeled wild-type oligonucleotides were able to compete with binding of the corresponding 32P-labeled wild-type oligonucleotides (Fig. 5a and b, lanes 1 to 3). This showed that the protein complex in the EMSA was due to specific binding of each of the core promoter regions to a protein or protein complex in the extract.

Mutated double-stranded oligonucleotides containing the core promoter of Piexo1 and Piendo1 were further used in the EMSA to determine if there was a correlation between protein binding and core promoter function, as determined in the transient assays. The same mutations (pEXO*+1/+3, pEXO*+9/+11, pEndO*+9/+11, and pEXO*+6/+8) that reduced GUS levels in transient expression assays (Fig. 2 and 3b) also reduced binding of the EMSA complex (Fig. 5). Mutations (pEXO*+6/+8 and pEndO*+2) that did not reduce GUS levels in the transient expression assays (Fig. 2 and 3b) also did not drastically reduce binding of the EMSA complex (Fig. 5). This strongly suggests that the complexes observed for Piexo1 and Piendo1 in the EMSA contain a sequence-specific DNA-binding protein or proteins that contact the core promoter regions of Piexo1 and Piendo1.

The band intensities produced in the EMSAs were measured using a PhosphorImager and the Molecular Dynamics Image Quant version 5.0 software. A correlation coefficient of −0.97 was obtained when the band intensities of the 400× competition lanes of the pEXO WT, pEXO*+1/+3, pEXO*+9/+11, and pEXO*+6/+8 oligonucleotides (Fig. 5a, lanes 3, 5, 7, and 9) were regressed against the relative percent GUS activities of the corresponding promoter constructs (Fig. 2). A correlation coefficient of −0.87 was obtained when the band intensities of the 100× competition lanes of the pEndO WT, pEndO*+9/+11, pEndO*+6/+8, and pEndO*+2 oligonucleotides (Fig. 5b, lanes 3, 5, 7, and 9) were regressed against the relative percent GUS activities of each corresponding promoter construct (Fig. 3b). The high correlation between the two different methods used to assay the effect of mutations within the 19-nt core promoter region confirms the importance of specific nucleotides within the core region.

DISCUSSION

We found that the core promoter regions of most oomycete genes sequenced by the summer of 2003 are comprised of a highly conserved 19-nt core region encompassing nucleotides −4 to +15 (Fig. 1). It includes the TSS in the vast majority of sequenced oomycete genes. Previously this core sequence had been identified as a 16-nt sequence (−4 to +12) (40). Sequence comparison, transient expression assays, and protein binding studies support the presence of two regions within the 19-nt region that are particularly important for core promoter function (Fig. 1, 2, 3, and 5). The first region (−2 to +5), encompassing the TSS, contains a known eukaryotic core promoter element, i.e., the initiator (Inr). The second region (+7 to +15) downstream and flanking the Inr, hereafter referred to as flanking promoter region (FPR), previously has not been identified as a region important for core promoter function in any other eukaryote.

The putative Inr of oomycetes shows striking similarities with the Inr of Trichomonas, Drosophila, and mammals in several aspects. First, as in mammals, Trichomonas, and Drosophila, the oomycete Inr function is highly dependent on the A at +1, on a T or A at +3, and on a pyrimidine at −1. Mutation of these residues results in the most severe reduction in promoter function (17, 28, 29; Fig. 3). Second, eukaryotic Inr elements overlap the TSSs of genes and direct accurate transcription initiation (47). In oomycetes the Inr also overlaps the TSSS of the genes, although we do not know if it directs accurate transcription initiation (Fig. 1). Thirdly, the oomycete Inr consensus sequence (Y-C-A-T-T-Y-Y, where Y = pyrimidine) is similar to that of mammals (Y-Y[C]-A-A-N-T/A-Y-Y), Drosophila (T-C-A-T-C-T-C), and Trichomonas (T-C-A-T-C/T-C/A) (17, 28; Fig. 1).

Although only a limited number of oomycete promoter regions are known, our current data suggest that a substantial...
(a) Competitor

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<th>Inr</th>
<th>FPR</th>
<th>Relative % GUS activity</th>
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<td>pEXO WT</td>
<td>CTCCTCTCA::TTTCCGAGAGCTGAGGTC</td>
<td>94 ± 8</td>
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<tr>
<td>pEXO+6/+8</td>
<td>CTCCTCTCA::TTTCCGAGAGCTGAGGTC</td>
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<tr>
<td>pEXO+9/+11</td>
<td>CTCCTCTCA::TTTCCGAGAGCTGAGGTC</td>
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(b) Competitor

<table>
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<tr>
<th>Inr</th>
<th>FPR</th>
<th>Relative % GUS activity</th>
</tr>
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<tbody>
<tr>
<td>pENDO WT</td>
<td>GGAAGAACGGCTCA::TTTCCCAAACCTATCTCCTCTCG</td>
<td>95 ± 8</td>
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<td>GGAAGAACGGCTCA::TTTCCCAAACCTATCTCCTCTCG</td>
<td>54 ± 13</td>
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number of oomycete genes could possibly contain a core promoter structure consisting of the Inn and FPR. However, as more sequence data become available more diversity might be detected in oomycete core promoters. Nonetheless, it is intriguing that P. infestans and Trichomonas (protist) are the only two known eukaryotes that contain a core promoter structure where the Inn is overrepresented in genes, and there is an apparent absence of TATA boxes (28, 42). Other eukaryotes such as plants, metazoans, and Drosophila have more diverse core promoter structures, with the Inn being less common. For example, in metazoans and plants only a relatively small subset of the known genes contains an Inn, with the majority of known genes containing TATA boxes (34, 45). In these eukaryotes, Inn-containing genes are present mostly as genes that are regulated and not as housekeeping genes (34, 46). In Drosophila, analyses of 250 promoters showed that approximately 26% of genes contain an Inn and 43% contain a TATA box (23).

In eukaryotes, transcription from Inn-containing, TATA-less promoters is not well characterized and several different models have been hypothesized that might apply to Inn recognition of promoters. The data that we have obtained from analyzing the P. infestans Inn-containing region. The Drosophila model proposes direct recognition by a general transcription factor (TFIID-associated factor) of the Inn and an adjacent downstream element called DPE (downstream promoter element) (3, 4, 23). In this model the DPE (positioned +28 to +32) functions cooperatively with the Inn, since a mutation in either the Inn or DPE abolishes promoter activity and binding of the general transcription factor TFII D (5). Similarly, in P. infestans we have found a putative sequence-specific element called FPR downstream from the Inn. Furthermore, as for Drosophila, mutations in either the P. infestans Inn or downstream element (FPR) result in a reduction of promoter function as well as a reduction in the binding of a protein complex detected in EMSA studies (Fig. 2, 3, and 5). This could suggest that, as in Drosophila, the oomycete Inn functions cooperatively with a downstream sequence-specific core promoter region, i.e., the FPR.

Our current knowledge of oomycete basal transcription is very limited. Future studies need to address important aspects of Inn function known to exist in other eukaryotes. For example, it is known that there are sequence-specific activator elements overlapping and adjacent to the Inn in some metazoan genes (10, 13). Furthermore, as more oomycete sequence data become available it will be important to carry out sequence and functional analyses of promoter regions to determine if other core promoter structures are represented in oomycetes. The identification of novel oomycete core promoter elements and transcription factors could provide targets for drugs to mitigate the harmful effects of this group of destructive pathogens.

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