Linkage between Mitochondrial Hypovirulence and Viral Hypovirulence in the Chestnut Blight Fungus Revealed by cDNA Microarray Analysis

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Received 17 June 2004/Accepted 9 August 2004

The phenomenon of transmissible hypovirulence (virulence attenuation) associated with biological control of natural populations of the chestnut blight fungus *Cryphonectria parasitica* can be experimentally reproduced by infection with hypovirus cDNA clones (viral hypovirulence) or by mutation of mitochondrial DNA (mtDNA) in the absence of virus infection (mitochondrial hypovirulence). We now report the use of an established *C. parasitica* cDNA microarray to monitor nuclear transcriptional responses to an mtDNA mutation of *C. parasitica* strain EP155, designated EP155/mit2, which was previously shown to induce elevated alternative oxidase activity and hypovirulence (C. B. Monterio-Vitorello, J. A. Bell, D. W. Fulbright, and H. A. Bertrand, Proc. Natl. Acad. Sci. USA 92:5935–5939, 1995). Approximately 10% of the 2,200 genes represented on the microarray exhibited altered transcript accumulation as a result of the mit2 mtDNA mutation. While genes involved in mitochondrial function were clearly represented in the EP155/mit2-responsive gene list, direct parallels to the well-characterized *Saccharomyces cerevisiae* retrograde response to mitochondrial dysfunction were not observed. Remarkably, 47% of the genes that were differentially expressed following the infection of strain EP155 by the prototypic hypovirus CHV1-EP713 had similarly changed transcript accumulation in the virus-free EP155/mit2 mutant. These results establish a linkage between viral and mitochondrial hypovirulence and raise questions regarding the relationship between hypovirus infection and mitochondrial dysfunction. The combined set of transcriptional profile data provides a foundation for future studies on mitochondrion-to-nucleus communications in the context of hypovirus infection and senescence associated with mitochondrial dysfunction in filamentous fungi.

The term “mitochondrial hypovirulence” refers to a cytoplasmically transmissible form of virulence attenuation (hypovirulence) in the chestnut blight fungus *Cryphonectria parasitica*, that is associated with mitochondrial defects (23). The term was introduced to distinguish this form of hypovirulence from the more commonly observed form caused by double-stranded RNA (dsRNA) viruses, most notably viruses in the family *Hypoviridae* (hypoviruses). Both viral hypovirulence and mitochondrial hypovirulence arose in and are associated with the biological control of natural *C. parasitica* populations (3, 8, 17, 24).

Fulbright et al. (14) reported the first isolation of a dsRNA virus-free hypovirulent strain of *C. parasitica* recovered from a healing canker in the Kellogg Forest, Mich. Subsequent studies (14, 23) revealed that this and other dsRNA-free hypovirulent *C. parasitica* strains collected from the same general geographic area exhibited high levels of alternative oxidase activity, a hallmark of mitochondrial dysfunction that is often associated with fungal senescence (15). Monterio-Vitorello and colleagues (23) were able to provide direct evidence that mutation of mitochondrial DNA (mtDNA) in the laboratory could induce transmissible hypovirulence. Conidia of the highly stable *C. parasitica* strain EP155 were exposed to UV mutagenesis, and mutants were selected for a cyanide (CN)-resistant respiratory phenotype. Subsequent characterization of these respiration-deficient mutants revealed both nuclear and mtDNA mutations. Nuclear mutants, e.g., cyt1 and cyt2, were similar in colony morphology and virulence to wild-type EP155. In contrast, mtDNA mutants, e.g., mit1 and mit2, exhibited a flat, highly pigmented, and irregular colony phenotype similar to that observed for the virus-free hypovirulent strains recovered from the field. Moreover, the mit1 and mit2 mutants were hypovirulent and the hypovirulence phenotype was transmissible by anastomosis.

The molecular biology of viral hypovirulence was recently advanced by the development of a spotted cDNA microarray platform containing ca. 2,200 *C. parasitica* genes. Allen et al. (1) used the cDNA microarray to show that infection by the prototypic hypovirus CHV1-EP713 results in a persistent re-programming of a significant portion (estimated at 13.4%) of the *C. parasitica* transcriptome. We now report the use of the *C. parasitica* cDNA microarray to examine changes in host transcript accumulation associated with mitochondrial hypovirulence. The analysis unexpectedly identified over 70 *C. parasitica* genes that were similarly altered in transcript accumulation in hypovirulent strains associated either with hypovirus infection or with mtDNA mutation, establishing a linkage between mitochondrial and viral hypovirulence.

**MATERIALS AND METHODS**

**Strains and media.** Hypovirus-free *C. parasitica* strain EP155 (ATCC 38755) and isogenic strain EP155/CHV1-EP713 (ATCC 52571), which is infected with the prototypic hypovirus CHV1-EP713 (27), were maintained on potato dextrose...
agar (PDA; Difco, Detroit, Mich.) at a temperature between 22 and 24°C with a 12-h light-dark cycle at 1,300 to 1,600 lx. Isogenic, hypovirus-free strains of EP155 containing mitochondrial (mit2; designated EP155/mit2 in this study) and nuclear (cyt2; designated EP155/cyt2) mutations were kindly provided by H. Bertani (University of California State University). The EP155/mit2 mutant (mutant 35.12 in reference 23) and EP155/cyt2 (mutant 181.3 in reference 23) mutants were generated by screening 4,393 random isolates initially for slow growth on solid medium and subsequently for alternative oxidase activity as an indication of mitochondrial dysfunction. The EP155/mit2 mutant was subsequently shown to retain a slow growth phenotype, producing flat, pigmented colonies with irregular margins. In contrast, EP155/cyt2 was subsequently shown to produce colonies with growth and morphological phenotypes similar to those of the wild-type strain EP155. The EP155/mit2 mutant was determined to contain a mitochondrial mutation based on transmission and inheritance and was clearly hypovirulent. Mutant EP155/cyt2 was determined to be the result of a nuclear mutation and was as virulent as the wild-type strain. However, both the EP155/mit2 and EP155/cyt2 mutants exhibited elevated levels of cyanide-resistant respiration. Cultures used for RNA preparations were grown on cellophane membranes overlaying PDA (cellophane-PDA) under the conditions described above.

Total RNA isolation. Cultures grown on PDA-cellophane for 6 days were harvested by freezing the mycelia in liquid nitrogen, with immediate grinding of the mycelia into a fine powder by use of a mortar and pestle. RNA isolation was performed as described previously (1).

Microarray slide printing. A second-generation spotted cDNA chip was constructed from the same C. parasitica expressed sequence tag (EST) library printing plates that were used to construct the previously profiled first-generation chips (1). For the second print run, GAPS II-coated slides from Corning (coated with gamma amino propyl silane) were substituted for poly-L-lysine slides prepared in-house because of their superior hybridization characteristics, low background, and longer shelf life. Purified PCR products were arrayed singly, with an average spot diameter of 100 μm and a spot spacing of 300 μm. After printing, the spotted cDNA was cross-linked, washed, and blocked as described previously (1).

Fluorescent probe generation for hybridization. Fluorescence-labeled cDNA probes were prepared from total RNA (25 μg per probe) by the direct incorporation of Cy3- or Cy5-dUTP by use of a CyScribe first-strand cDNA labeling kit (Amersham Pharmacia) primed with oligo(dT) according to the manufacturer's instructions. Unincorporated nucleotides were removed by using a Microcon-30 spin column and probes were processed according to the method of Allen et al. (1).

Microarray hybridization and scanning. Prehybridization, hybridization, and posthybridization wash steps were performed as suggested by the manufacturer of the GAPS II slides (Corning). Each hybridized chip was scanned in both the Cy3 and Cy5 channels with an Affymetrix 418 scanner as described previously (1).

Microarray data processing and analysis. Data processing and analysis were performed as described by Allen et al. (1), with the exception that differentially expressed genes were identified as spots with log2 ratios of ≥2 standard deviations from the average log2 ratio of each data set in a minimum of three of four hybridizations instead of four of six hybridizations. Measurements for control cDNAs spotted on the microarray chips to monitor hybridization performance are reported in supplemental Tables 1 to 3 (see the URLs below). The expected measurements were obtained for all control cDNAs (1, 2). Control cDNA spots designated as “not scored” (NS) in the supplemental Tables 2 and 3 were appropriately not scored as differentially expressed because they did not satisfy the mathematical requirement of having a log2 ratio of ≥2 standard deviations from the average log2 ratio of the data set.

Microarray data management. To ensure that independent lists of differentially expressed genes reflected biological differences rather than technical differences associated with different microarray print runs, we repeated the hybridizations described by Allen et al. (1) for a hypovirus-free C. parasitica strain (EP155) and C. parasitica infected with the prototypic hypovirus CHV1-EP713. Consequently, all profiling results reported here are from hybridizations performed on the same set of second-generation chips. Comparisons between multiple lists and subsequent curation of differentially expressed clone lists were performed as described previously (2).

Nonredundant lists for hybridizations involving EP155/CHV1-EP713 versus EP155, EP155/mit2 versus EP155, and EP155/cyt2 versus EP155 are available at http://www.umbi.umd.edu/~cbf/nuss/2G155-713NonRedundant.pdf (supplemental Table 1), http://www.umbi.umd.edu/~cbf/nuss/155-mit2NonRedundant.pdf (supplemental Table 2), and http://www.umbi.umd.edu/~cbf/nuss/155-cyt2NonRedundant.pdf (supplemental Table 3), and lists of genes identified as being commonly differentially expressed in two or more strains of C. parasitica are available at http://www.umbi.umd.edu/~cbf/nuss/Overlap713-mit2NonRedundant.pdf (supplemental Table 4), http://www.umbi.umd.edu/~cbf/nuss/OverlapMit2Cyt2NonRedundant.pdf (supplemental Table 5), http://www.umbi.umd.edu/~cbf/nuss/OverlapCyt2713NonRedundant.pdf (supplemental Table 6), and http://www.umbi.umd.edu/~cbf/nuss/Mit2Cyt2713Overlap.pdf (supplemental Table 7).

Validation of EP155/mit2 versus EP155 differentially expressed clones by real-time RT-PCR. A total of 10 clones that were predicted to be differentially expressed in EP155/mit2 and EP155 were tested by real-time reverse transcription-PCR (RT-PCR) by use of an Applied Biosystems (Foster City, Calif.) GeneAMP 5700 sequence detection system and an Applied Biosystems TaqMan RT kit. The reaction conditions used were described previously (1). Transcript abundance was calculated by the comparative ΔCt method (20) relative to the amount of 18S rRNA in the sample, with primers and conditions as described previously (25). Differential expression based on real-time RT-PCR measurements was defined as a change in transcript accumulation of twofold or more.

RESULTS

Transcriptional profiling of mitochondrial (EP155/mit2) and nuclear (EP155/cyt2) C. parasitica mutants selected for alternative oxidase activities. The EP155/mit2 and EP155/cyt2 mutants (Fig. 1) were derived from a genetic screen (23) designed to select for C. parasitica strains that exhibit mitochondrial dysfunction. Mutant EP155/mit2 was subsequently determined to contain a mitochondrial mutation and was clearly hypovirulent, while the EP155/cyt2 mutant was determined to be the result of a nuclear mutation and was as virulent as the wild-type strain. Changes in C. parasitica nuclear gene expression in response to the mit2 and cyt2 mutations were monitored in this study by use of a custom microarray chip similar to that previously used to monitor changes in C. parasitica transcript accumulation as a result of hypovirus infection (1, 2).
Of the ca. 2,200 *C. parasitica* genes represented on the microarray, 210 were scored as being differentially expressed in the EP155/mit2 mutant relative to the wild-type strain EP155 (124 upregulated and 86 downregulated; see Materials and Methods and supplemental Table 2). These changes contrasted significantly with those observed for the EP155/cyt2 mutant, for which only 51 of the 2,200 *C. parasitica* genes were scored as being differentially expressed relative to the wild-type EP155 control strain (Fig. 2). In this case, the majority of the differentially expressed genes showed a reduction in transcript accumulation (40 downregulated and only 11 upregulated; see Materials and Methods and supplemental Table 3). An examination of the fold change values for the two mutant strains presented in supplemental Tables 2 and 3 also indicated that the changes in transcript accumulation for the EP155/cyt2 mutant were generally of a lower magnitude than those for the EP155/mit2 mutant.

An inspection of the lists of genes that were differentially expressed as a result of the mit2 and cyt2 mutations revealed 34 genes in common (see Materials and Methods and supplemental Table 5) (Fig. 2). Thus, 67% of the genes that were differentially expressed as a result of the cyt2 mutation also had altered transcript accumulation in the EP155/mit2 mutant strain. However, only four of the genes on the common list had altered transcript accumulation in the same direction. That is, 30 of the genes that were differentially expressed in both mutants had altered transcript accumulation in opposite directions. The number of genes on the common list represented only 16% of the 210 genes that were scored as differentially expressed in EP155/mit2.

Comparison of transcription profiles between *C. parasitica* strains exhibiting mitochondrial and viral hypovirulence. Monteiro-Vitorello et al. (23) noted several phenotypic similarities between mitochondrial hypovirulent and viral hypovirulent *C. parasitica* strains. Both hypovirulent strains generally grew slower, produced fewer conidia, and formed abnormal colony morphologies relative to their corresponding virulent strains. While these general similarities held true for the EP155/mit2 and the CHV1-EP713-infected EP155 strain (EP155/CHV1-EP713), as indicated in Fig. 1, the colony morphologies of these two strains appeared quite dissimilar. The EP155/mit2 mutant produced colonies with a considerable level of orange pigmentation and very irregular margins, while the colonies produced by EP155/CHV1-EP713 were white, with only slightly irregular margins. Nevertheless, both EP155/mit2 and EP155/CHV1-EP713 have significantly reduced virulence (23). The availability of a list of genes that are differentially expressed as a result of the mit2 mutation provided an opportunity to test for possible molecular linkages between mitochondrial hypovirulence and viral hypovirulence.

A total of 73 genes were found to be differentially expressed in both the EP155/mit2 mutant and EP155/CHV1-EP713 (see Materials and Methods and supplemental Table 4) (Fig. 2), representing 47.4% of the total nonredundant CHV1-EP713-responsive genes. Even more remarkably, 42 of the 73 genes were found to be upregulated in both hypovirulent strains, while 29 were found to be downregulated in both strains (Fig. 3B). Only 2 of the 73 genes on the common list were regulated in opposite directions.

A comparison of the lists of genes that were differentially expressed in the EP155/cyt2 mutant and EP155/CHV1-EP713 revealed only 24 common genes (see Materials and Methods and supplemental Table 6) (Fig. 2). However, similar to the observation for the EP155/cyt2 and EP155/mit2 common gene list, only 1 of the 24 was regulated in the same direction in both strains (AEST-32-B-01). Twenty genes were differentially regulated in both mutant strains and CHV1-EP713-infected EP155 (see Materials and Methods and supplemental Table 7). For 18 of the 20 genes on the three-strain common list, transcript accumulation was altered in the same direction for EP155/mit2 and EP155/CHV1-EP713 but in the opposite direction for EP155/cyt2. One gene, AEST-04-D-04, was upregulated in EP155/mit2 and EP155/cyt2 and downregulated by CHV1-EP713 infection, while the gene AEST-32-B-01 was downregulated in all three strains.

A hierarchical clustering of the average log2 (Cy3/Cy5) ratios for the cDNA clones spotted on the microarray chip across the hybridization data sets for the two mutant strains and strain EP155/CHV1-EP713 relative to the set for strain EP155 provided a visual summary of the similarities among the expression change profiles generated in this study (Fig. 3A). The similarities in the direction and magnitude of change for the genes that were commonly altered in transcript accumulation by the mit2 mutation and CHV1-EP713 infection are quite apparent by comparing columns 1 and 2. Comparing column 3 with columns 1 and 2 illustrates the generally lower magnitude

![Venn diagram illustrating the total number of unique differentially expressed genes within each hybridization and between hybridizations. Each complete circle represents hybridization results between EP155/mit2 and EP155, EP155/CHV1-EP713 and EP155, and EP155/cyt2 and EP155. The blue region indicates the number of commonly differentially expressed genes (73) found between EP155/mit2 versus EP155 and between EP155/CHV1-EP713 versus EP155. A list of these genes can be found in supplemental Table 4. The yellow region indicates the number of commonly differentially expressed genes (34) found between EP155/mit2 versus EP155 and between EP155/cyt2 versus EP155. A list of these genes can be found in supplemental Table 5. The orange region indicates the number of commonly differentially expressed genes (24) found between EP155/CHV1-EP713 versus EP155 and between EP155/cyt2 versus EP155. A list of these genes can be found in supplemental Table 6.](image-url)
of change in transcript accumulation caused by the cyt2 mutation. Additionally, column 3 reflects the observation that most of the changes in the transcript abundance of commonly altered genes were in the opposite direction for the cyt2 mutant relative to the changes caused by the mit2 mutation or CHV1-

EP713 infection. The clustering of the 73 genes that were commonly altered in expression in EP155/mit2 and EP155/CHV1-EP713 (lanes 4 and 5, respectively, in Fig. 3B) clearly illustrates the high level of coordination in the transcriptional response for these genes in EP155/CHV1-EP713 and EP155/mit2.

As in previously reported microarray analyses with the custom C. parasitica microarray chip, the transcript accumulation of several control cDNAs spotted onto the chip was measured to provide an internal check of consistency between hybridizations (values are available in supplemental Tables 1 to 3). Additionally, real-time RT-PCR was used to confirm the microarray results for 10 genes that were scored as being differentially expressed in both the EP155/mit2 mutant and EP155/CHV1-EP713. Differential expression was confirmed for 9 of the 10 genes, with the fold change values presented in Table 1, consistent with the validation rates of \( \approx 90\% \) reported earlier (1, 2).

**DISCUSSION**

Viruses, mutant mtDNA, and a mitochondrial plasmid have all been implicated in the attenuation of virulence (hypovirulence) for several phytopathogenic fungi (reviewed recently in reference 3). Definitive evidence for mycovirus-mediated transmissible hypovirulence was provided by the construction of full-length infectious cDNA clones of hypoviruses associated with hypovirulence in C. parasitica (6, 7). A role for mitochondrial dysfunction and/or modified mtDNA in the attenuation of fungal virulence was strongly supported by the demonstration that experimentally induced cytoplasmic mutations of a virus-free C. parasitica strain conferred the transmissible hypovirulence phenotype (23). The results presented in this report establish a molecular genetic linkage between mitochondrial hypovirulence and viral hypovirulence.

Microarray analyses of nucleus-encoded transcriptional changes accompanying mtDNA mutations and mitochondrial dysfunctions have not previously been reported for filamentous fungi, and little is known about the communication between organelles in these organisms. In the budding yeast Saccharomyces cerevisiae, nuclear gene expression has been shown to respond to changes in mitochondrial function through a Rtg1/Rtg2/Rtg3-dependent signaling pathway termed the retrograde response (22). Epstein et al. (13) recently expanded the view of nuclear transcriptional responses to mitochondrial dysfunction in S. cerevisiae by performing a genome-wide microarray analysis of respiration-deficient petite cells. Twenty-two genes represented on the C. parasitica cDNA microarray correspond (with E values of \( \approx 10^{-15} \)) to yeast genes that were scored as being differentially expressed in the study by Epstein et al. (13). Of these, only three were scored as being differentially expressed in the EP155/mit2 mutant. These included homologues to genes encoding AAC1, an ADP-ATP translocator (AEST-08-D-04; upregulated in EP155/mit2), the heat shock protein HSP104 (AEST-14-A-02; downregulated in EP155/mit2), and YNL134C, a quinone oxidoreductase (AEST-31-G-08; upregulated in EP155/mit2). The homologue of CIT2 (E value = \( 10^{-65} \); AEST-48-E-06), a gene encoding peroxisomal citrate synthase and commonly used as a diagnostic marker of retrograde regulation (22), did not have altered transcript accumulation in EP155/mit2. Genes that were not listed in the S. cerevisiae study but that correspond to genes with mitochon...
drial functions, e.g., those encoding NADH-cytochrome b5 reductase (Mcr1; AEST-04-C-01; E value = 2 × 10^{-53}) (21) and cytochrome c (CYC1; AEST-38-D-11; E value = 3 × 10^{-50}) (19), were found to be upregulated in EP155/mit2 (7- and 3.5-fold, respectively). Thus, while there is little evidence to support a response similar to the yeast retrograde response, C. parasitica nucleus-encoded genes with mitochondrial functions have clearly altered transcript accumulation as a result of the mit2 mutation. Additional studies of these responses are expected to uncover novel mitochondrion-to-nucleus signaling pathways for filamentous fungi.

The changes in the transcriptional profile associated with the cyt2 mutation were clearly less extensive (51 genes for EP155/cyt2 versus 210 for EP155/mit2) and generally of a lower magnitude than those observed for EP155/mit2 (Fig. 2 and 3). These differences were consistent with the differences in the severity of the phenotype and in virulence levels for the two mutants (Fig. 1) (23). However, the fact that 34 of the 51 cyt2-responsive genes also had altered transcript accumulation in EP155/mit2 suggests that the nuclear mutation in the EP155/cyt2 strain and the cytoplasmic mutation in the EP155/mit2 strain result in modifications in some common regulatory pathway(s). Interestingly, the two mutations appear to modify this common pathway(s) in opposite directions: 30 of the 34 common genes were upregulated in EP155/mit2 and downregulated in EP155/cyt2. Moreover, 20 of the 34 genes that were responsive to both mit2 and cyt2 mutations were also responsive to CHV1-EP713 infection, with 18 of those genes being upregulated in EP155/mit2 and EP155/CHV1-EP713 and downregulated in EP155/cyt2.

Parallels between mitochondrial hypovirulence in C. parasitica and well-characterized fungal senescence syndromes in the nonpathogenic filamentous fungi Neurospora crassa and Podospora anserina have been extensively reviewed (4, 5). Similarities include reduced levels of cytochromes, elevated alternative oxidase activities, aberrant growth phenotypes, female sterility, the cytoplasmic transmissibility of respiratory defects (suppressiveness), and the gradual accumulation of plasmid-like DNA in mtDNA fractions resembling senDNAs, which are associated with senescing cultures of other filamentous fungi. It will be of considerable interest, and also highly informative, to compare the nuclear transcriptional responses of the hypovirulence-associated mtDNA mutation reported here with the responses of senescing strains of N. crassa and P. anserina when they become available.

The remarkable observation that 47% of the genes that were scored as being differentially expressed by CHV1-EP713 infection in this study also had altered transcript accumulation as a result of a mtDNA mutation that induces transmissible hypovirulence clearly establishes a molecular linkage between mitochondrial hypovirulence and viral hypovirulence. This linkage is further supported by the fact that of the 73 genes that were scored as being differentially expressed in EP155/CHV1-EP713 and EP155/mit2, 42 were upregulated in both strains, 29 were downregulated in both strains, and only 2 were regulated in opposite directions (AEST-04-D-04 and AEST-06-B-03) (Table 1; Fig. 3B). The high degree of similarity in the coordinated transcriptional response for this set of genes is even more surprising in light of the very different morphological changes caused by hypovirus infection and mtDNA mutation (Fig. 1), suggesting that these common changes may be related to the shared phenotypic trait of virulence attenuation. An apparent connection between hypovirus infection and mitochondrial dysfunction is particularly intriguing given the well-established role of mitochondrial defects in fungal senescence (reviewed in reference 4) and the growing associations between mitochondrial dysfunction and a multitude of human diseases, including diabetes (i.e., aberrant sugar metabolism) (reviewed in reference 12).

One straightforward interpretation of the striking similarities in transcriptional profile changes for two strains exhibiting viral and mitochondrial hypovirulence is that the hypovirus CHV1-EP713 directly causes mitochondrial dysfunction. However, altered mitochondrial function is not a characteristic that is generally observed for hypovirus-infected C. parasitica strains (4). Additionally, there is no direct correlation between an elevated alternative oxidase activity and virulence attenuation, i.e., both the EP155/cyt1 and EP155/cyt2 nuclear mutants exhibited alternative oxidase activity but retained virulence (23). An alternative interpretation of the similarities in transcriptional responses is that hypovirus infection and mitochondrial dysfunction modulate a common regulatory pathway.

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**TABLE 1. Real-time PCR measurements of 10 representative genes that are differentially expressed in both EP155/mit2 and EP155/CHV1-EP713 relative to EP155**

| Clone ID | Fold change (avg) in transcript abundance relative to EP155* | Putative identification | E value |
|----------|-------------------------------------------------------------|------------------------|---------|
| mit2     | CHV1-EP713                                                  |                        |         |
| 13-1     | +68.6                                                      | C. parasitica positive control | 0.0     |
| AEST-04-D-04 | +16.8                                               | Unknown               | >10^{-2}|
| AEST-05-B-03 | +12.7                                               | TOXD protein          | 3 x 10^{-19}|
| AEST-06-B-03 | +17.5                                               | CGI-83 protein        | 3 x 10^{-30}|
| AEST-11-F-11 | -3.7                                                | Chain A. alpha-1,2-mannosidase | 8 x 10^{-33}|
| AEST-13-A-03 | +24.3                                               | Cytochrome P450 monoxygenase | 6 x 10^{-63}|
| AEST-25-F-08 | +54.6                                               | Unknown               | >10^{-2}|
| AEST-32-C-09 | -1.3                                                | Mst12                 | 6 x 10^{-65}|
| AEST-11-B-06 | -4.3                                                | Hypothetical protein  | 2 x 10^{-12}|
| AEST-38-H-09 | +188.4                                              | Glutathione S-transferase | 1 x 10^{-38}|

* Averages were calculated from six measurements taken from two independent RNA preparations. †, measurements were previously confirmed and reported by Allen et al (1); †, measurements were previously confirmed and reported by Allen et al (2). The differential expression of AEST-30-C-09 in EP155/mit2 relative to EP155 (underlined value) failed to be confirmed by realtime RT-PCR.
This could be by direct alteration of the same pathway or by modulation of separate pathways that feed into a common response network. In this regard, an altered intracellular redox environment has previously been implicated by microarray studies which revealed increased glutathione S-transferase transcript accumulation in *C. parasitica* infected with CHV1-EP713 (1). The amount of glutathione S-transferase was also strongly increased (Table 1, AEST-38-H-09) in the mitochondrial mutant profiled here, which hints at the possibility that reactive oxygen species may contribute to the etiology of hypovirulence.

The possibility that various pathways in cellular respiration are similarly altered in viral and mitochondrial hypovirulence is further suggested by the observation that phosphoglucomutase (AEST-36-G-11; see supplemental Table 4), a key regulatory enzyme controlling entry into glycolysis, is down-regulated in EP155/CHV1-EP713 and EP155/mit2. Since glycolysis represents the first stage in the eventual production of ATP through the catabolism of glucose, the downregulation of phosphoglucomutase is likely to result in altered ATP levels. The TOR signaling pathway provides one possible candidate for mediating several of the responses mentioned above. The TOR pathway integrates cellular responses to starvation, mitochondrial dysfunction, ATP levels, and osmotic stress and is conserved in all eukaryotes (9–11).

The availability of the *C. parasitica* cDNA microarray platform provides the means of examining whether the common responses to CHV1-EP713 infection and mitochondrial hypovirulence extend to other viruses that are associated with fungal virulence attenuation. These include other species within the family *Hypoviridae* (28), a *C. parasitica* reovirus (16), and the *C. parasitica* mitovirus (26), which is localized to the mitochondria and related to viruses that are associated with a hypovirulence phenotype in other plant pathogenic fungi, e.g., the Dutch Elm disease fungus *Ophiostoma ulmi* (18). It is anticipated that such studies will provide new insights into relationships between virus infection, mitochondrion-to-nucleus communications, and virulence in pathogenic filamentous fungi.

**ACKNOWLEDGMENTS**

We thank Helmuth Bertrand for providing the mutant strains EP155/mit2 and EP155/cyt2 and for information regarding their history and characterization. This study was supported in part by Public Health Service grant GM55981 to D.L.N.

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