Mitochondrial Functions Mediate Cellulase Gene Expression in *Trichoderma reesei*†

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ABSTRACT: We examined the effects of inhibition of mitochondrial functions on the expression of two nuclear genes encoding the extracellular cellobiohydrolase I (*cbh1*) and endoglucanase I (*egl1*) of the cellulase system of the filamentous fungus *Trichoderma reesei*. The *cbh1* and *egl1* transcripts are repressed at a low oxygen tension, and by glucose at a concentration known to repress mitochondrial respiration. The transcripts are also down-regulated by chemical agents known to dissipate the proton electrochemical gradient of the inner mitochondrial membrane and blocking of the electron-transport chain, such as DNP and KCN, respectively. These results suggest that expression of those transcripts is influenced by the physiological state of the mitochondria. In addition, heterologous gene fusion shows that the sensitivity of the expression of those transcripts to the functional state of the mitochondria is transcriptionally controlled through the 5′-flanking DNA sequence of those genes.

In eukaryotes, the mitochondria play a key role in the control of the metabolic status of the cell. Located within this organelle are the majority of the enzymes required for the oxidation of pyruvate and other fuels by the citric acid cycle, the electron-transfer system of the respiratory chain, the complex that synthesizes ATP, the enzymes required in the biosynthesis of numerous amino acids, and the heme biosynthetic enzymes.

It is well established that, in the yeast *Saccharomyces cerevisiae*, the nucleus plays an important role in the maintenance and assembly of the mitochondria and in regulation of the expression of the mitochondrial genome (Attardi & Schatz, 1988; Tzagoloff & Myers, 1986). However, the role of the mitochondria in regulating nuclear gene expression is still poorly understood. Several studies suggested that the mitochondria can communicate with the nucleus, influencing the expression of certain nuclear genes encoding mitochondrial and nonmitochondrial proteins (Parikh et al., 1987; Shyjan & Butow, 1993; Siemens et al., 1980). These studies pointed to the importance of the functional state of mitochondria in the regulation of this path of communication from mitochondria to nucleus (Shyjan & Butow, 1993).

Repression of mitochondrial respiration in yeast cells, induced either by drugs or by limitation of oxygen or heme (Siemens et al., 1980; Forsburg & Guarente, 1989), has been shown to affect the expression of nucleus-encoded cytochrome *c*. Recently, the effect of perturbation of mitochondrial function on the expression of two nuclear genes encoding the mitochondrial and the peroxisomal forms of the citrate synthase in *Saccharomyces cerevisiae* was reported (Liao et al., 1991; Liao & Butow, 1993). In these works, it was shown that elevation of the level of the transcript of the peroxisomal isoform of the citrate synthase enzyme was obtained as a result of alteration of the mitochondrial function by the addition of respiratory inhibitor drugs; the transcript was also increased in mutant [rho°] cells in which the mitochondrial DNA was entirely deleted or in cells containing a disruption of the mitochondrial form of the citrate synthase.

Glucose, the most convenient carbon energy source for microorganisms and one of the most important effectors of metabolism, also plays an important role in the physiological and metabolic status of mitochondria. In *S. cerevisiae*, the presence of more than 5 mM glucose in the culture medium was shown to block mitochondrial respiratory capacity, and, under this altered metabolic situation, yeast cells satisfy all of their energy requirements by glycolysis (Perlman & Mahler, 1974).

In this work, we investigated the interaction between the mitochondria and the nucleus and whether such regulation takes place in microorganisms other than the unicellular *S. cerevisiae*. To that end, we analyzed whether the functional and metabolic status of the mitochondria could influence the expression of two nuclear genes encoding the extracellular cellobiohydrolase I (CBHI) and endoglucanase I (EGI) of the cellulolytic system in the multicellular fungus *Trichoderma reesei*.

T. reesei is a filamentous fungus able to utilize cellulose as a carbon energy source (Montenecourt, 1983). The utilization of cellulose by this microorganism is achieved by an extracellular cellulase system that catalyzes the hydrolysis of cellulose to glucose (Coughlan & Ljungdahl, 1988). Cellulose-degrading enzymes of *T. reesei* are among the best characterized; this cellulolytic system consists of three major classes of enzymes: cellobiohydrolases (CBHI and CBHII), which cleave cellobiosyl units from the nonreducing end of cellulose chains; endoglucanases (EGI and EGII), which cleave internal glucosidic bonds; and β-glucosidases (Barnett et al., 1991), which cleave cellooligosaccharides to produce glucose [for a review, refer to Béguin (1990)].

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In *T. reesei*, the expression of the cellulase genes is stringently regulated by the carbon source. Growth on cellulose results in at least a 1200-fold induction of the *cbh1* and *egl1* transcripts (Urioste et al., unpublished data). This induction by cellulose appears to require basal expression of cellulase that is needed to catalyze the formation of a soluble inducer from cellulose (El-Gogary et al., 1989; Kubicek, 1987). The cellulase genes are also subjected to a regulatory circuit of catabolite repression, where glucose strongly represses the expression of the *cbh1* transcript (El-Gogary et al., 1989).

In this report, we show that the *cbh1* and *egl1* transcripts are down-regulated by oxygen limitation in the culture media, by chemical agents known to inhibit the mitochondrial activity, such as DNP and KCN, and by glucose at a concentration known to repress mitochondrial respiration. In addition, we present data suggesting that the sensitivity of the expression of those transcripts to the functional and metabolic state of the mitochondria is transcriptionally controlled through the 5′-flanking DNA sequence of those genes.

EXPERIMENTAL PROCEDURES

Materials. [γ-^{32}P]ATP and [α-^{32}P]dCTP (specific radioactivity: 3000 Ci/mmol) were purchased from Amersham Corp. Avicel (PH101, microcrystalline cellulose) was generously provided by Forlab-Kelrio S/A, Brazil. The RNA inhibitor Thiolutin was obtained from Pfizer Inc., Groton, CT. 4-Methylumbelliferyl β-D-glucuronide (MUG) and 4-methylumbelliferone (MU) were purchased from Sigma.

Inoculum and Culture Conditions. Maintenance of *T. reesei* cultures, inocula preparation, culture medium, and washing of Avicel were carried out as previously described (El-Gogary et al., 1989). Mycelia from germinated spores were centrifuged, washed twice with 100 mM potassium phosphate buffer (pH 6.0), and incubated on a rotary shaker for 2 h. Mycelia (2 mg dry weight) were suspended in culture medium lacking glycerol, and the inducer, cellulose (1%), was added to the reaction mixtures as indicated. In aerated condition, cultures were incubated on a rotary shaker (200 rpm) at 28 °C for the indicated time. Under this condition, the concentration of dissolved O_2 was 4 mg/L, representing a saturation of approximately 57%. In static condition (low oxygen tension), cultures were incubated without shaking at 28 °C where dissolved [O_2] was 0.15 mg/L, corresponding to a saturation of about 2.1%.

The concentration of dissolved oxygen was measured using a polarographic oxygen electrode (Digimed DM0/1) obtained from Digimed Instrumentação Analítica LTD do Brasil.

The effects of the uncoupler of oxidative phosphorylation, DNP (2,4-dinitrophenol), and of the inhibitor of the electron-transport chain, KCN, on the expression of the cellulase transcripts were examined by addition of these chemical agents (final concentration of 1 mM) to cells induced with cellulose for 20 h; then aliquots were withdrawn, as indicated, and filtered, and cells were frozen in liquid nitrogen.

The RNA polymerase inhibitor Thiolutin (Jimenez et al., 1973; Herrick et al., 1990) was dissolved in dimethyl sulfoxide at 2 mg/mL and was added to cellulose-induced culture samples at a final concentration of 6 μg/mL. Aliquots were removed at the indicated time and filtered, and the cells were frozen in liquid nitrogen. All cells were kept at −70 °C until used for RNA extraction.

RNA Isolation and Analyses. Frozen mycelium was ground to a fine powder under liquid nitrogen, and total RNA was isolated as described by Chirgwin et al. (1979). RNAs (10 μg) were separated by electrophoresis on a 1.2% agarose gel after denaturation with glyoxal and dimethyl sulfoxide (Sambrook et al., 1989), and transferred to a Hybond-N membrane. DNA probes were labeled with [α-^{32}P]dCTP using the random primers DNA labeling system (Gibco-BRL). Membranes were hybridized with radiolabeled probes as indicated. The *cbh1* and *egl1* probes used in hybridization were the *Eco*RI DNA fragment (720 bp) or the *Kpn*I−*Pst*I DNA fragment (1050 bp) containing part of the coding region of the *cbh1* or *egl1* genes, respectively (Pentillä et al., 1986; Shoemaker et al., 1983). For *cbh2* and *egl2* probes, the entire coding sequences, from Gln 1 to Lys 397 for *egl2* and from Gln 1 to Leu 447 for *cbh2* (Saloheimo et al., 1988; Teeri et al., 1987), were amplified using the polymerase chain reaction (PCR) in the presence of *T. reesei* genomic DNA and specific primers. The plasmid pEUC-1 containing 1.1 kb of *Sal*I DNA fragment from the *T. reesei* actin gene was used as a probe (Matheucci et al., 1995).

Construction of Plasmids and Fusion Genes. The plasmid pCB-GUS-1.6 contains a 1.6 kb *Sac*II−*Xba*I DNA fragment of the 5′ region of the *cbh1* gene (the *Sac*II site is located at position −10 relative to the ATG codon, with the A being position 0) fused to the *E. coli* β-glucuronidase (GUS) gene. To construct pCB-GUS-1.6, an *Eco*RI−*Sma*I DNA fragment containing the β-glucuronidase gene including the 3′ termination region from the nopaline synthase (NOS) gene was isolated from pB121 (Jefferson et al., 1986) and inserted into the *Eco*RI−*Sma*I sites of Bluescript KS and designated pBS-GUS. A 1.6 kb *Sac*II−*Xba*I DNA fragment of the 5′ region of the *cbh1* gene, which was treated with T4 DNA polymerase at the *Sac*II site prior to generation of the *Xba*I site, was inserted into the *Sma*I−*Xba*I site of pBS-GUS, generating pCB-GUS-1.6.

Transformation of T. reesei. Protoplast preparation and transformation were achieved according to the method of Penttilä et al. (1988). The plasmid pCSN43 containing the gene coding for hygromycin B resistance, controlled by the promoter and the terminator of the *trpC* gene of *A. nidulans* (Cullen et al., 1987), was used as a selection marker. Transformants were purified by single-spore selection on media containing 200 μg/mL hygromycin B.

Enzyme Activities. Cellulase activity was measured using Avicel (microcrystalline cellulose) as a substrate as described previously (El-Gogary et al., 1989). β-Glucuronidase activity was measured using 4-methylumbelliferyl β-D-glucuronide (MUG) as a substrate (Jefferson, 1987). The assay mixture contained (final volume of 500 μL) 50 mM Na_2HPO_4, pH 7.0, 10 mM β-mercaptoethanol, 10 mM EDTA, 0.1% sodium laurylsarcosine, 0.1% Triton X-100, and 1 mM MUG. After addition of *T. reesei* extract (10−50 μL), samples were incubated at 37 °C, and aliquots (100 μL) were removed at 30−60 min intervals into tubes containing 0.9 mL of Na_2CO_3. The relative fluorescence of the product, 4-methylumbelliferone (MU), was measured using a Perkin-Elmer LS-5 luminescence spectrometer (excitation at 365 nm and emission at 455 nm). Transformed *T. reesei* cells were extracted in the assay mixture mentioned earlier except that MUG was omitted.

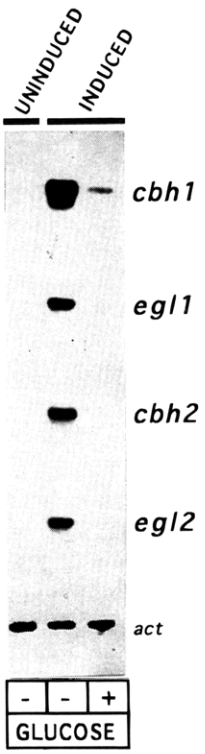

FIGURE 1: Effect of glucose on the expression of the cellulase mRNAs. *T. reesei* cells were grown on 0.8% glycerol (un-induced) or induced with cellulose (1%) for 20 h, then glucose was added (1% final) as indicated, and total RNA was isolated after an additional 1 h. Aliquots containing 10 μg of RNA were fractionated electrophoretically on a 1.2% agarose gel, transferred to Hybond-N membranes, and hybridized with labeled probe as indicated (for details, refer to Experimental Procedures). Induction of the cellulase transcripts by cellulose and repression by glucose were performed under aerated conditions (4 mg of oxygen/L).

RESULTS

Induction of the Cellulase Transcripts by Cellulose. Expression of the cellulase system of *T. reesei* requires induction with cellulose. *T. reesei* produces at least two cellobiohydrolases, CBHI and CBHII (Shoemaker et al., 1983; Teeri et al., 1987; Chen et al., 1987), and two endoglucanases, EGI and EGII (Penttilä et al., 1986; Saloheimo et al., 1988; Van Arsdell et al., 1987). The members of this system act synergistically to catalyze the hydrolysis of cellulose to oligosaccharides (Henrissat et al., 1985). Figure 1 shows that the transcripts of the cellulase system are not detected when cells are grown on glycerol. However, the system is induced when cellulose is used as a carbon source and repressed by the addition of 55 mM glucose (1%). Actin (*act*) is included as a control and presents no significant alteration. Thus, we decided to analyze further the effect of glucose on the expression of the *cbh1* and *egl1* transcripts, the major members of the cellulolytic system.

The time course of cellulose-induced *cbh1* and *egl1* mRNA was examined. Transcripts were not detected before 12 h of induction; thereafter, accumulation of the transcripts was achieved, reaching their fully induced levels after 16 h of induction (Figure 2, inset). It is worth mentioning that, in a separate experiment, we found that the kinetics of induction of those transcripts were the same between 12 and 16 h, suggesting that those transcripts are coordinately expressed; in the above-mentioned experiments, actin transcript presented no alteration (results are not shown). Low basal

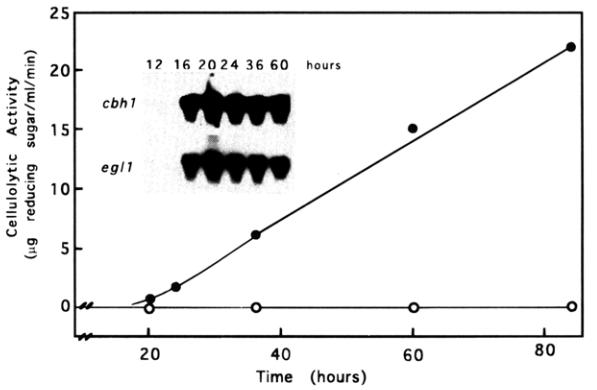

FIGURE 2: Effect of cellulose on the level of *cbh1* and *egl1* mRNAs and extracellular cellulase activity. *T. reesei* cells were grown on cellulose (●) or glycerol (○), and cellulase activity was measured in the culture media as described under Experimental Procedures. mRNAs were analyzed as described in Figure 1.

expression of the cellulase system was implicated in triggering the expression of the cellulase transcripts; this basal expression is needed to catalyze the formation of a soluble inducer from cellulose (El-Gogary et al., 1989; Kubicek, 1987). Most probably, the time lag (12−16 h) of the inductive process of the cellulase transcripts is due to the time required to catalyze the formation of the soluble inducer from cellulose, which in turns triggers the expression of the transcripts.

To show that accumulation of cellulase mRNAs resulted in induction of extracellular cellulase activity, we determined cellulase activity in the culture medium during the inductive process (Figure 2). The results indicated that cellulase-induced mRNAs were associated with cellulase synthesis and secretion into the culture medium. It is interesting to note that there is a 4 h lag between the detection of the cellulase transcripts and the appearance of the cellulase activity in the culture medium. This time lag, besides the time necessary for the secretion processes, is probably due to the interaction between the enzyme proteins and the cell wall prior to total secretion to the culture medium.

During the inductive process, we found that the external glucose concentration fluctuated between 5 and 25 μM (results are not shown); this concentration is far below the minimum required for glucose repression (see later). Most probably, the glucose formed from cellulose hydrolysis is used rapidly by the microorganism as a carbon energy source.

Effect of Glucose and Oxygen on Expression of cbh1 and egl1 Transcripts. As shown earlier, in the presence of the inducer (cellulose), glucose causes repression of the cellulase system; conversely, the expression of cellulase requires glucose starvation and the presence of inducer. In this experiment, we examined the expression of cellulose-induced *cbh1* and *egl1* transcripts during metabolic switch from glucose starvation to glucose feeding. *T. reesei* cells were induced with cellulose at 28 °C and aerated (4 mg/L O_2) on a rotary shaker at 200 rpm for 20 h (Figure 3A). Increasing amounts of glucose were added to induced cultures which were either incubated under aerated conditions (4 mg/L O_2) (Figure 3B) or left static (nonaerated, 0.15 mg/L O_2) (Figure 3C) for an additional 60 min. The results presented in Figure 3B show that, under aerated conditions (4 mg/L O_2), there is no effect on the expression of *cbh1* and *egl1* transcripts until the glucose concentration exceeds 1 mM. Repression was particularly apparent when 5.5 mM glucose (0.1%) was

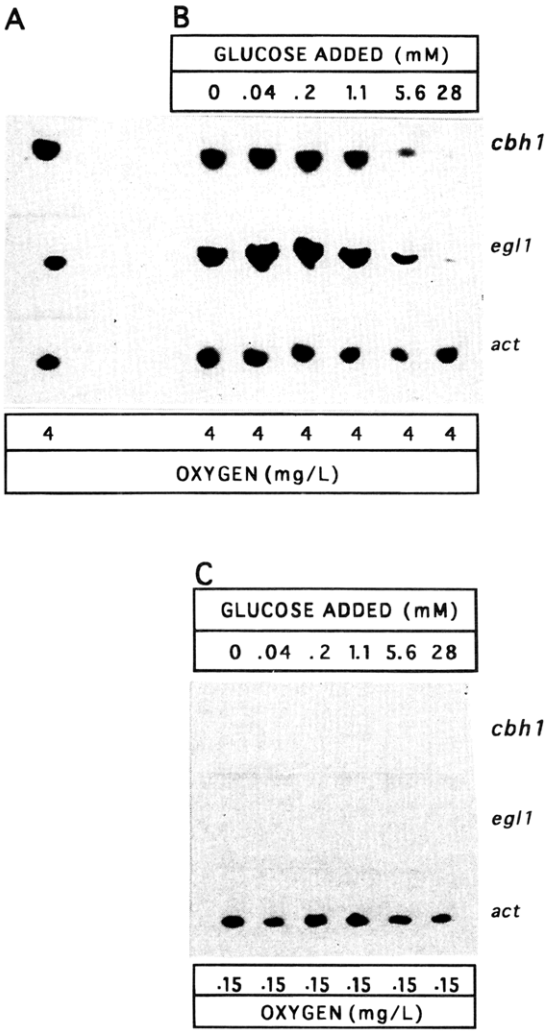

FIGURE 3: Effect of glucose concentration and oxygen tension on the level of *cbh1* and *egl1* mRNAs. mRNAs were isolated and analyzed from *T. reesei* cells grown on cellulose under different conditions of oxygen tension as indicated. (A) RNA was isolated and analyzed after 20 h. (B and C) As in (A), except that after 20 h glucose was added as indicated and RNAs were analyzed after an additional hour.

added to the culture medium. In contrast, in the absence of glucose, the *cbh1* and *egl1* transcripts were not detected 1 h after oxygen dropped to 0.15 mg/L in the induced culture media (Figure 3C, first lane). However, when the Northern blot (Figure 3C, first lane) was exposed for a longer period of time, the *cbh1* and *egl1* transcripts were detected and represented not more than 15% of those transcripts measured in RNA isolated from aerated induced *T. reesei* culture (the result of longer exposure is not shown; see later Figure 4, lanes 1−3). The actin transcript (*act*) was unaffected by glucose repression or oxygen limitation and is presented as a control.

Effect of Inhibitors of Mitochondrial Oxidative Phosphorylation on the Expression of cbh1 and egl1 Transcripts. The repression of the cellulase transcripts is observed at a concentration of glucose (5 mM) which was reported to cause respiration repression in yeast (Perlman & Mahler, 1974). Given that the decrease in oxygen tension also resulted in repression of the cellulase transcripts, we decided to examine the effect of chemical agents known to dissipate the proton electrochemical gradient of the inner mitochondrial membrane and to block the electron-transport chain, such as DNP

KCN	-	-	-	-	-	+	+
DNP	-	-	-	+	+	-	-
Oxygen (mg/L)	4	.15	.15	4	4	4	4
Time (min)	0	30	60	30	60	30	60

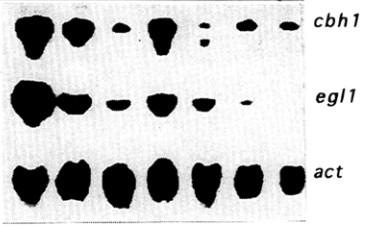

FIGURE 4: Effect of KCN and DNP on the level of *cbh1* and *egl1* mRNAs. *T. reesei* cells were grown on cellulose under an oxygen tension of 4 mg/L. After 20 h (taken as zero time in the table), *T. reesei* cells were kept under one of following conditions: oxygen tension of 0.15 mg/L; oxygen tension of 4 mg/L; and KCN or DNP (final concentration of 1 mM) were added. Samples were removed at 30 and 60 min intervals, and RNAs were isolated and analyzed as indicated.

and KCN, respectively, on the expression of those transcripts under aerated conditions. To that end, cells were induced with cellulose for 20 h under aerated conditions, and then DNP or KCN (1 mM) were added to aliquots which were subjected to 30 and 60 min of aerated conditions. The results show that the expression of *cbh1* and *egl1* transcripts was indeed repressed in cells exposed to DNP or KCN (Figure 4). Actin transcript was included as a control and shows no significant alteration. It is important to note that the half-lives of the *egl1* and *act* transcripts are similar (see later); therefore, the observed repression of the cellulase transcripts by KCN and DNP is specific for the cellulase transcripts and is not the result of cell inviability due to the addition of those inhibitors. In support of this conclusion, we observed no difference in the growth rate between cells exposed to DNP for 3 h and a control experiment (no added DNP) after switching to a medium containing no DNP.

Comparison of egl1 mRNA Decay Rates Measured after Glucose Repression with Decay Rates after Inhibition of Transcription with Thiolutin. The effect of glucose on the decay rates of the *egl1* transcripts was measured and compared with the decay rates measured after inhibition of transcription with Thiolutin. The results presented in Figure 5 indicate that no significant differences were observed in the decay rates of the *egl1* transcript whether transcription was inhibited by Thiolutin or cells were catabolically repressed by glucose. Similar results were obtained with the *cbh1* transcript (data not shown). Interestingly, actin transcript, which is unaffected by glucose in the presence of Thiolutin, was found to have a decay rate similar to the catabolically repressed *egl1* transcript. The results indicate that glucose repression of the *egl1* and *cbh1* transcripts is not controlled by a process involving mRNA stability.

5′-Flanking cbh1 DNA Sequence Controls Glucose Repression and DNP Down-Regulation in a Heterologous Gene Construct. To investigate whether glucose repression and DNP down-regulation of the *cbh1* transcript are due to transcriptional or posttranscriptional events, we analyzed a heterologous gene construct in which the bacterial β-glucuronidase gene was placed under the control of the *cbh1* promoter. The plasmid pCB-GUS-1.6 contains a 1.6 kb DNA fragment of the 5′ region of the *cbh1* gene fused to the *E. coli* β-glucuronidase (GUS) gene and the 3′ termina-

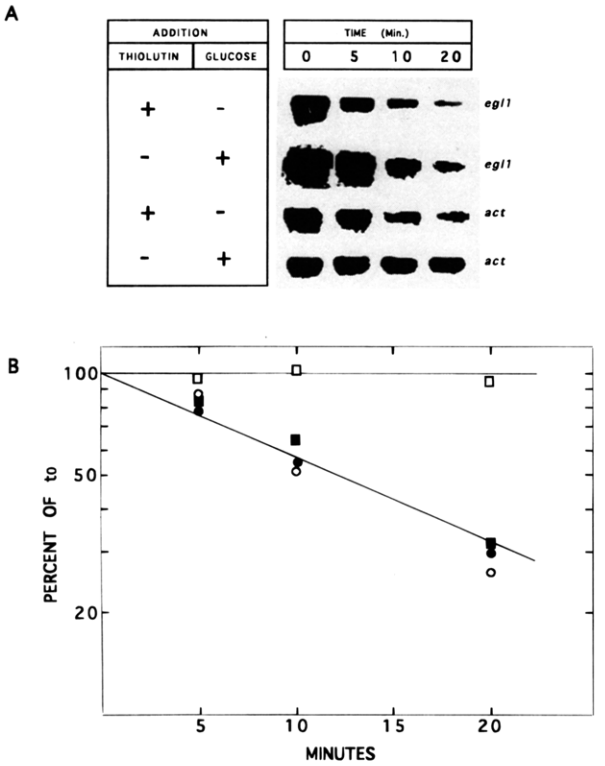

FIGURE 5: mRNA decay in Thiolutin-treated and glucose-treated cells. (A) *T. reesei* cells were grown on cellulose for 20 h and then treated with Thiolutin (6 μg/mL) or with glucose (55 mM). RNAs were isolated and analyzed as indicated. (B) Autoradiograms for RNA blots shown in panel A were measured by densitometry; the data are plotted as the percentage of RNA remaining versus time of incubation in the presence of Thiolutin (actin, ■; egl1, ●) or glucose (actin, □; egl1, ○). Values represent averages of three different analyses; variations did not exceed 10%.

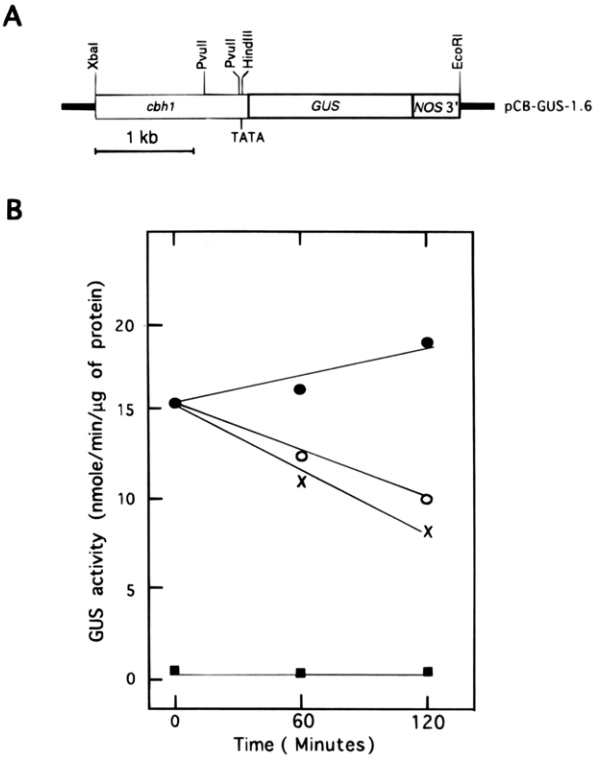

FIGURE 6: Control of glucose repression and DNP down-regulation in a heterologous gene construct by a 5′-flanking *cbh1* DNA region. (A) The plasmid pCB-GUS-1.6 contains a 1.6 kb DNA fragment of the 5′ region of the *cbh1* gene fused to the *E. coli* β-glucuronidase gene (GUS) and the 3′ termination region of the nopaline synthase (for details, refer to Experimental Procedures). (B) *T. reesei* cells were transformed with pCB-GUS-1.6, and a stable transformant, FLAVO 1.6-GUS, was isolated. FLAVO 1.6-GUS strain was induced with cellulose or grown in the presence of glycerol, at 28 °C on a rotary shaker at 200 rpm (4 mg/L O_2). After 18 h of induction, glucose or DNP (final concentration, respectively, 55 mM glucose or 1 mM DNP) was added (time 0 min) to aliquots which were subjected to an additional 60 and 120 min of aerated condition. GUS activity was then determined in the following aliquots: grown on glycerol (■); cellulose (●); cellulose plus DNP (○); and cellulose plus glucose (×). Values represent averages of three different experiments; variations did not exceed 10%.

tion region of the nopaline synthase (NOS) (Figure 6A; for details, refer to Experimental Procedures).

T. reesei cells were cotransformed with pCB-GUS-1.6 vector DNA (Figure 6A) and DNA from pCSN43, a vector conferring resistance to the drug hygromycin B (Cullen et al., 1987). Stable transformed cells were purified by three rounds of single-colony isolation under continuous drug selection. A *T. reesei* transformant was isolated, FLAVO 1.6-GUS, and found to be resistant to hygromycin B after three passages on nonselective media. Total DNA from this transformant was isolated, and Southern analysis showed that the plasmid, pCB-GUS-1.6, was integrated in the fungal genome into a site other than the *cbh1* locus (results are not shown).

FLAVO 1.6-GUS strain was induced with cellulose or grown in the presence of glycerol at 28 °C on a rotary shaker at 200 rpm (4 mg/L O_2) for 18 h, and then glucose or DNP (final concentration of 55 mM glucose or 1 mM DNP) was added to induced aliquots which were subjected to aerated conditions for an additional 60 and 120 min. Figure 6B shows that no GUS activity was observed in extracts of cells grown in the presence of glycerol, whereas cellulose stimulates transcription and subsequent expression of GUS activity. These results indicate that the FLAVO 1.6-GUS transformant harboring the integrated GUS gene under the control of the *cbh1* promoter expresses GUS activity in a manner resembling that of the *cbh1* gene; i.e., cellulose stimulates expression whereas glycerol has no effect. However, induced cells exposed to glucose or DNP for 2 h resulted in down-regulation of the GUS activity by about 50%. These results indicate that glucose repression and DNP down-regulation of the *cbh1* gene are transcriptionally controlled through its 5′-flanking DNA sequences.

DISCUSSION

It has recently become apparent that the metabolic status and the integrity of organelles, mitochondria, or chloroplasts can influence nuclear gene expression (Parikh et al., 1987; Taylor, 1989; Shyan & Butow, 1993). In *S. cerevisiae*, the transcript of the peroxisomal isoform of the citrate synthase enzyme is increased in a respiratory-deficient mutants or after inhibition of the oxidative phosphorylation activity in a respiratory-competent strain (Liao et al., 1991; Liao & Butow, 1993). In human cells, inhibition of mitochondrial function with DNP leads to down-regulation of the nuclearly-encoded ADP/ATP translocase transcripts (Lunardi & Attardi, 1991). In plants, photooxidative damage of chloroplasts results in down-regulation of expression of nuclear genes encoding chloroplast and nonchloroplast proteins (Taylor, 1989; Susek & Chory, 1992; Mayfield, 1990). This regulatory process in which the cell adjusts to changes in

mitochondrial activities by influencing nuclear gene expression is called retrograde communication (Parikh et al., 1987; Shyan & Butow, 1993).

In this study, we provide evidence showing that interaction between the mitochondria and the nuclear genome takes place in the multicellular fungus *T. reesei*; this interaction is involved in regulation of the nuclear genes (Mäntylä et al., 1992) encoding the extracellular CBHI and EGI of the cellulase system in this microorganism. Down-regulation of the *cbh1* and *egl1* transcripts was observed when the oxygen tension in the culture medium was decreased, or after the addition of 5.5 mM glucose (0.1%). Interestingly, this concentration of glucose is known to cause repression of mitochondrial respiration in *Saccharomyces cerevisiae* (Perlman & Mahler, 1974). Our results also show that inhibition of the electron-transport chain by KCN or dissipation of the proton electrochemical gradient of the mitochondrial membrane by DNP results in down-regulation of the *cbh1* and *egl1* transcripts.

To examine further the effect of inhibition of the mitochondrial activity on the expression of *cbh1* and *egl1* transcripts, we determined whether the sensitivity of the expression of those transcripts to the functional status of the mitochondria is due to transcriptional or posttranscriptional events. The effect of glucose and the transcriptional inhibitor Thiolutin on the turnover of the *egl1* transcript was analyzed. Using either the transcriptional inhibitor Thiolutin (Jimenez et al., 1973; Herrick et al., 1990) or glucose, our results show that, in both cases, the half-life of the *egl1* transcript is similar. In addition, the actin mRNA, which is not catabolically repressed by glucose, was found to have a half-life similar to that of *egl1*. These results indicate that glucose repression of the *egl1* transcript is not regulated posttranscriptionally. The result of heterologous gene fusion between the *cbh1* promoter and the *E. coli* β-glucuronidase gene shows that glucose repression and DNP down-regulation of the *cbh1* gene are controlled transcriptionally by 5′-flanking *cbh1* DNA sequences.

Our results show that inhibition of the mitochondrial function results in down-regulation of the two transcripts of the cellulase system, *cbh1* and *egl1*; conversely, these transcripts are expressed when *T. reesei* cells are grown in the presence of the inducer (cellulose) and at a glucose concentration in which the mitochondria are active. In light of these data, it is possible to suggest a role of the mitochondria in regulating the expression of the cellulase transcripts. In cells growing under aerated conditions and in the presence of cellulose and the absence of glucose, cellulase transcripts are expressed and the enzymes are secreted to the extracellular medium to hydrolyze cellulose to glucose. The liberated glucose is then oxidized by the tricarboxylic acid cycle (TCA) to supply the needed energy. However, if the extracellular glucose concentration exceeds that which affects mitochondrial activity by repressing respiration, the cellulase transcription will turn off. Also, cellulase transcripts will turn off with a decrease in the metabolic activity of the cells provoked by limiting oxygen in the culture medium. Therefore, it seems that the mitochondria also function as a sensitive organelle for detecting the availability of energy fuel in the cell and regulate the expression of nuclear genes, which in turn modulate the metabolic status of the cell.

Although the work presented in this report indicates that, in *T. reesei*, the metabolic status and the activity of the mitochondria could influence expression of the cellulase genes, the nature of the signal generated by the mitochondria and the mechanism by which this signal alters cellulase gene expression remain to be established. One possible signal is the fluctuation of the level of some metabolic intermediate of the TCA cycle or the accumulation of a specific metabolite which might mediate the activity of the transcription complex. In this regard, we detected no change in the concentration of cAMP after addition of glucose. Moreover, we tested the effect of protein kinase inhibitors, staurosporine and H7 [1-(5-isoquinolinylsulfonyl)-2-methylpiperazine], on the expression of the cellulase transcripts. Our results (data not shown) indicate that those inhibitors have no effect on either the expression of the *cbh1* or *egl1* transcripts in the presence of cellulose or the repression of these transcripts by glucose.

In *S. cerevisiae*, heme serves as an intermediate in the signaling mechanism for oxygen levels (Zitomer & Lowry, 1992). Some enzymes involved in the synthesis of heme are oxygenases; therefore, heme level is controlled by oxygen tension. An example of a gene regulated by heme at the transcriptional level is the iso-1-cytochrome *c* (*CYC1*) of *S. cerevisiae*. The *CYC1* promoter contains DNA regulatory sites including a binding sequence for the transcriptional activator HAP1, a heme-dependent activator (Forsburg & Guarente, 1989). Most of the heme-regulated genes are those encoding respiratory functions, oxidative damage repair, and the utilization of oxygen in membrane and heme biosynthesis [for a review, refer to Zitomer and Lowry (1992)]. Although cellulases are hydrolases catalyzing the cleavage of β-1,4-glycosidic bonds in cellulose and are not involved in the above-mentioned processes, further work will be required to determine whether heme serves as an intermediate for the influence of oxygen on the expression of the cellulase transcripts.

In summary, our results demonstrate that in *T. reesei*: (a) the transcripts of the nuclear genes encoding the extracellular cellulase system are coordinately induced by cellulose and repressed by glucose; (b) the *cbh1* and *egl1* transcripts are down-regulated by perturbation of mitochondrial function, including limited oxygen in the culture media, chemical agents known to inhibit the mitochondrial activity, and glucose at a concentration known to repress respiration; (c) the sensitivity of the expression of these transcripts to the functional state of the mitochondria is transcriptionally controlled through the 5′-flanking DNA sequences of those genes.

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