The *Aspergillus fumigatus* celllobiohydrolase B (cbhB) promoter is tightly regulated and can be exploited for controlled protein expression and RNAi

Michael Bromley, Caroline Gordon, Nuría Rovira-Graells & Jason Oliver

F2G Ltd, Lankro Way, Eccles, Manchester, UK

Correspondence: Michael Bromley, F2G Ltd. Lankro Way, Eccles, Manchester, M30 0BH, UK. Tel.: +44 161 785 1276; fax: +44 161 785 1273; e-mail: mikebromley@f2g.com

Received 7 July 2006; revised 23 August 2006; accepted 24 August 2006. First published online 19 September 2006. DOI:10.1111/j.1574-6968.2006.00462.x

Editor: Bernard Prior

Keywords

*Aspergillus fumigatus*; cbhB; celllobiohydrolase; RNAi; promoter.

Abstract

The utility of the *Aspergillus fumigatus* celllobiohydrolase cbhB promoter for controlled gene expression has been investigated. cbhB message was present at high levels in the presence of carboxymethylcellulose and undetected in the presence of glucose. A reporter construct using the cbhB promoter showed similar behaviour and gave lower message levels than the *Aspergillus nidulans* alcA promoter under repressing conditions. An RNAi construct driven by the cbhB promoter was used to down-regulate the alb1 gene; transformants showed low alb1 message levels and a loss-of-function phenotype with carboxymethylcellulose, while both wild-type message levels and phenotype were seen with glucose. The cbhB promoter is therefore tightly controlled and can be exploited for the study of *A. fumigatus*.

Introduction

In recent years, the filamentous fungus *Aspergillus fumigatus* has become a significant cause of infection in humans and as such has become the focus of much study (Latge, 2001; Marr *et al*., 2002). It is thought to be the leading mould pathogen in leukaemia and transplant patients and is responsible for mortality in a large number of individuals with immunological disorders. Until quite recently, the ‘gold standard’ treatment for aspergillus infection has been the intravenous application of amphotericin B. However, significant side-effects, coupled with a poor treatment outcome, have lead to the search for more efficacious and less toxic drugs. The available alternatives, e.g. voriconazole and itraconazole, show little improvement in outcome but do have fewer side-effects (Denning, 1996; Herbrecht *et al*., 2002). However, isolates resistant to these azoles have been identified (Mosquera & Denning, 2002). Current antifungal drugs are directed against a narrow range of pathways and proteins and the identification of novel targets is critical in the search for new antifungal drugs. One aspect of this is the development of new target validation technologies. In particular, improved methods for controlled regulation of genes may be used to investigate essentiality and therefore determine whether a gene is suitable as a drug target.

Several inducible promoter systems have been developed in filamentous fungi, namely the glaA, cbh1, alcA, thiA and tet systems (Keranen & Penttilä, 1995; Siedenberg *et al*., 1999; Romero *et al*., 2003; Mouyna *et al*., 2004; Shoji *et al*., 2005; Vogt *et al*., 2005); however, significant expression occurs in repressed or ‘off’ states. The development of an inducible system with improved repressing capabilities is therefore important to enable more complete analysis of essential genes, particularly those expressed at low levels. Here, we report studies of a number of *A. fumigatus* promoters and show that the promoter for the celllobiohydrolase cbhB is tightly controlled, showing high message levels in the presence of carboxymethylcellulose and low-to-undetectable levels of message in the presence of glucose. This represents a substantial improvement over other systems. We also show that the cbhB promoter is suitable for protein expression and inducible RNAi studies.

Materials and methods

*Aspergillus strains and culture conditions*

All methods described used *A. fumigatus* clinical isolate AF293 (NCPF7367) available from the NCPF at the Health Protection Agency, Bristol, UK. Conidia were collected from...
4- to 7-day-old cultures grown on Sabouraud Dextrose agar flasks at 37 °C.

**Construction of the expression plasmids and modified *A. fumigatus* strains**

All primers used for plasmid construction are given in Table 1. The cbhB promoter and terminator were amplified from *A. fumigatus* genomic DNA. Primers cbhBp_f and cbhBp_r were used to amplify a 1.2-kb fragment from −1 bp of the ATG codon from the cbhB gene (P<sub>cbhB</sub>). This fragment was cloned into the EcoRI site of puc19. Primers cbhBt_f and cbhBt_r were used to amplify a 0.8 kb from +1 of the TAG stop from the cbhB gene (T<sub>cbhB</sub>). This fragment was cloned into the XbaI site of the puc19-P<sub>cbhB</sub> plasmid. The hygromycin selectable marker hph was amplified from the pAN7.1 vector using primers hyg_f and hyg_r and cloned into the NarI site of the puc19-P<sub>cbhB</sub>-T<sub>cbhB</sub> plasmid to give plasmids pMJB104 and pMJB107 (Fig. 1).

The *Escherichia coli lacZ* gene was amplified from pDE1 (Fungal Genetics Stock Centre) using primers lacZ-XhoI_f and lacZ-XhoI_r and cloned into the XhoI site of the pMJB104 and pMJB107 expression cassettes immediately downstream of the promoter to act as a reporter gene, giving plasmids pMJB110 and pMJB201, respectively (Fig. 1). The reporter cassettes were introduced into *A. fumigatus* AF293 protoplasts to generate strains AF110-1 and AF110-2 from plasmid pMJB110 and AF201-1 and AF201-2 from plasmid pMJB201.

0.9 kb of the *Aspergillus nidulans* alcA promoter was amplified from plasmid pAL3 (Waring et al., 1989).

### Table 1. Primers used in this study

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence</th>
<th>Enzyme</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasmid construction</td>
<td></td>
<td></td>
</tr>
<tr>
<td>alcApF</td>
<td>GAATTTCGTCTGGGCCACAATAGACT</td>
<td>EcoRI</td>
</tr>
<tr>
<td>alcApR</td>
<td>GGTACCTTAAAGGTTGATATCGGTGGGGCA</td>
<td>KpnI</td>
</tr>
<tr>
<td>cbhBp_f</td>
<td>CAATTGCACCCAGCCTATGCATG</td>
<td>MunI</td>
</tr>
<tr>
<td>cbhBp_r</td>
<td>CAATTGCGGTAGGGAAGGTGAGGGCC</td>
<td>MunI</td>
</tr>
<tr>
<td>cbhBt_f</td>
<td>TCTAGAGCTAGCATGGGAGTGGG</td>
<td>XbaI</td>
</tr>
<tr>
<td>cbhBt_r</td>
<td>ACTAGTCCAGGGGACTGTGGTCA</td>
<td>SpeI</td>
</tr>
<tr>
<td>hyg_f</td>
<td>GGGCCCATCGATGTCATGGACCAGGTGACT</td>
<td>NarI</td>
</tr>
<tr>
<td>hyg_r</td>
<td>GGGCCCAAGAAGGATATTAATCTAAACA</td>
<td>NarI</td>
</tr>
<tr>
<td>lacZ-XhoI_f</td>
<td>CTCTAGATGGTCTTTTACAACTGCTGAC</td>
<td>XhoI</td>
</tr>
<tr>
<td>lacZ-XhoI_r</td>
<td>CTCTAGATTTATATTTTTGACCA</td>
<td>XhoI</td>
</tr>
<tr>
<td>RNAi_alb1_1_ant2_F</td>
<td>TTGACGAACAGCACGTCGTGAC</td>
<td>XbaI</td>
</tr>
<tr>
<td>RNAi_alb1_1_ant2_R</td>
<td>TTGACGAACAGCACGTCGTGAC</td>
<td>XbaI</td>
</tr>
<tr>
<td>RNAi_alb1_1_sense2_F</td>
<td>TTGACGAACAGCACGTCGTGAC</td>
<td>SacI</td>
</tr>
<tr>
<td>RNAi_alb1_1_sense2_R</td>
<td>TTGACGAACAGCACGTCGTGAC</td>
<td>SacI</td>
</tr>
<tr>
<td>RNAi_EGFP_R</td>
<td>TTGACACTCCAGCTGATGCGG</td>
<td>XbaI</td>
</tr>
<tr>
<td>RNAi_EGFP_F</td>
<td>TTGACACTCCAGCTGATGCGG</td>
<td>XbaI</td>
</tr>
<tr>
<td>RT PCR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACT470F</td>
<td>TGGTGTTCATCACGCTGTTCC</td>
<td></td>
</tr>
<tr>
<td>ACT960R</td>
<td>TCTATAGGAGGAGGAGAAGGG</td>
<td></td>
</tr>
<tr>
<td>CBHAF</td>
<td>CTCTCTACTCTGTCGAC</td>
<td></td>
</tr>
<tr>
<td>CBHAR</td>
<td>GGCAACGTGGAGACAGAGT</td>
<td></td>
</tr>
<tr>
<td>CBHB</td>
<td>GCTCAAGCGTGCTGATC</td>
<td></td>
</tr>
<tr>
<td>CBHB</td>
<td>GGCTAGGCTTGTAGAACC</td>
<td></td>
</tr>
<tr>
<td>INVBF</td>
<td>TGCTGCTTCTTCTATC</td>
<td></td>
</tr>
<tr>
<td>INVBR</td>
<td>TGCTGCTTGAGCGTCTAG</td>
<td></td>
</tr>
<tr>
<td>Real-time PCR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RtalbF</td>
<td>GGAGGATGGTCTAGGGCCAG</td>
<td></td>
</tr>
<tr>
<td>RtalbR</td>
<td>TCTAGGTGGTATGCTG</td>
<td></td>
</tr>
<tr>
<td>RttubBF</td>
<td>GCTCACTCTTCTGTCG</td>
<td></td>
</tr>
<tr>
<td>RttubBR</td>
<td>AGCAGGGTGAGTAAGCT</td>
<td></td>
</tr>
<tr>
<td>RtcbHF</td>
<td>TTGATGTCATGTCGTTCAAA</td>
<td></td>
</tr>
<tr>
<td>RtcbHR</td>
<td>GGCTGAGGCTTGTAGAACC</td>
<td></td>
</tr>
<tr>
<td>RtlacZF</td>
<td>ATCCCTCTGATGTCAGTG</td>
<td></td>
</tr>
<tr>
<td>RtlacZR</td>
<td>TATTGGCTCATTCCACCA</td>
<td></td>
</tr>
</tbody>
</table>

*For primers including restriction enzyme sites, these are indicated by underlined bases and the enzymes given in the ‘enzyme’ column.

†*MunI* has an overhang compatible with *EcoRI*.

‡*XbaI* and *SpeI* produce compatible overhangs.
using primers alcApF and alcApR and cloned directionally into the puc19 plasmid at the EcoRI and KpnI sites. The E. coli lacZ gene was amplified as before but with AflII linkers and introduced downstream of the alcA promoter. Finally, the hygromycin resistance marker was introduced into the NarI site as for the cbhB cassette to create palcA-LacZ (Fig. 1).

The alb1 RNAi construct was produced in a manner similar to that described in Liu et al. (2002). A sense and antisense alb1 fragment corresponding to 497 bp of the gene commencing at position 5142 bp from the start codon were amplified from A. fumigatus genomic DNA. Primers RNAi_alb1_sense2_F and RNAi_alb1_sense2_R with SacI and Cfr9I linkers, respectively, were used to amplify the sense fragment and primers RNAi_alb1_ant2_F, and RNAi_alb1_ant2_R, which are the same as the sense primers but with XbaI linkers, were used to produce the antisense fragment. A buffer fragment was obtained using primers RNAi_EGFP_F and RNAi_EGFP_R to amplify a 0.1-kb fragment of the enhanced green fluorescent protein (EGFP) gene from the pGFP-zeo plasmid. These fragments were sequentially cloned into pMJB107 to give plasmid pALBR2 (Fig. 1). This cassette was introduced into A. fumigatus AF293 protoplasts to generate strains ALBR2 A-D.

HindIII linearized plasmids were introduced into A. fumigatus AF293 by transformation of protoplasts (D’Enfert, 1996). Transformants were screened by PCR and only those strains with complete expression cassettes were used in further studies. For analysis of cbhB and alcA expression cassettes, all transformant strains were confirmed to be single copy by Southern analysis (data not shown).

β-galactosidase assay

1 × 10⁶ conidia mL⁻¹ were inoculated into 50 mL of Vogel’s media, 1% w/v glucose. Cultures were incubated for 14 h at 37°C with shaking at 220 r.p.m. Biomass was collected and 0.3 g wet weight was used to inoculate Vogel’s media supplemented with either 1% w/v glucose or 1% w/v carboxymethylcellulose (CMC). Cultures were incubated for 2, 4, 6 and 8 h at 37°C with shaking at 220 r.p.m. for CMC cultures and 4 h only for the glucose cultures.

Fifty milligrams wet weight biomass was placed in tubes containing DNA lysing matrix (Bio 101) with 1 mL protein extraction buffer (50 mM NaH₂PO₄ pH 7.0, 5 mM EDTA, 10 mM β-mercaptoethanol, 25 g mL⁻¹ PMSF), and samples were processed in a fastprep FP120 (Bio 101) for 20 s at speed five. Sample were cooled on ice for 5 min, processed
again as before and the lysates were placed on ice for 20 min. Protein extracts were separated from cellular debris by centrifugation and stored in 200 µL aliquots at −80 °C.

The β-galactosidase assay was performed in 96-well plates using 100 µL sample or standard (E. coli β-galactosidase, Sigma) in protein extraction buffer and 100 µL ortho-nitrophenol-β-D-galactoside (2 mg mL⁻¹). Samples were incubated at 37 °C for 1 h and then read at 415 nm.

Reverse Transcription-PCR analysis

1 × 10⁶ conidia mL⁻¹ were inoculated into 50 mL of Vogel’s media with 1% w/v glucose, 1% w/v sucrose or 1% carboxymethylcellulose. Total RNA was extracted using the FastRNA kit (QBIogene) following the manufacturer’s instructions. cDNA was synthesized from 1 µg RNA using an Avian Myeloblastosis Virus-Reverse Transcriptase kit (Promega) with random hexamers. Semi-quantitative PCR was carried out using appropriate dilutions of cDNA and primers; actin, ACT470F and ACT960R; invB, INVBF and INVBR; cbhA, CBHAF and CBHAR; and cbhB, CBHBF and CBHBR (Table 1). All PCRs comprised 30 cycles of 95 °C 1 min, 55 °C 1.5 min and 72 °C 1 min.

Real-time PCR analysis

cDNA was prepared as described above. The growth conditions were as described in β-galactosidase assay, with samples taken after 4 h. Reactions were carried out in an iCycler thermal cycler fitted with an iQ real-time PCR Detection System (BioRad) running iCycler iQ Optical System software (version 3a, BioRad). PCRs were typically performed in triplicate in 96-well plates with each 25 µL reaction containing 12.5 µL iQ SYBR Green Super-mix (BioRad), 5 pmol of each primer (Table 1) and 50 ng template cDNA. PCR conditions were 3 min at 95 °C then 60 cycles of 30 s at 95 °C, 30 s at 55 °C and 15 s at 72 °C. Fluorescence was measured at the end of the annealing step. Average Ct points were calculated and the ΔΔCt method (Pfaffl, 2001) was used to calculate the relative expression represented by the equation:

\[ \frac{E_{\Delta\DeltaCt\text{LacZ} \text{repressed-induced}}}{E_{\Delta\DeltaCt\beta\text{-tubulin repressed-induced}}} \]

Results

Regulation of the cellobiohydrolase and invertase genes

Many of the fungal cellulases and invertases are known to be regulated by carbon catabolite repression, via the creA-mediated pathway. Initial experiments assessed the expression of candidate genes under conditions thought likely to induce and repress.

Semi-quantitative PCR was used to assess the expression of the invertase gene invB (Afu2g01240) from mycelia grown in Vogel’s media using either 1% glucose or 1% sucrose as the sole carbon source. At the 12-h time-point, products were detected for invB at cDNA dilutions down to one in 5000 in the presence of sucrose and one in 500 in the presence of glucose, indicating a 10-fold difference in invB expression on sucrose compared with glucose, with expression still clearly visible on glucose (Fig. 2a). No difference could be seen in the glucose and sucrose samples by the 16 and 24 h time-points (data not shown). Similar analysis was
conducted for the invA gene (Afu6g05000) but no difference could be seen at any time-point (data not shown).

Levels of mRNA for cbhA (Afu6g07070) and cbhB (Afu6g11610) were determined for cultures grown in Vogel's media with either 1% glucose or 1% CMC as the sole carbon source. Expression of both genes was detected at 16 h in cultures grown with CMC, the cbhA gene being expressed c. 50-fold higher than the cbhB gene (Fig. 2b). No message could be detected for cbhB in the presence of glucose at 16 h even after extending the number of PCR cycles to 60. Low levels of expression, representing an approximate 1000-fold reduction on the CMC grown levels, were detected for cbhA in glucose-grown cultures. Similar results were seen at 24 h (data not shown). Insufficient biomass was obtained at the 12 h time-point to provide analysis. The promoter of the cbhB gene therefore showed optimal expression characteristics and was chosen for the development of an inducible system.

**Reporter gene analysis of the cellobiohydrolase B promoter**

In filamentous fungi, transcriptional cis-acting elements are generally thought to be restricted to 1 kb upstream of the initiation codon (Punt et al., 1995). Therefore, 1.2 kb of the cbhB upstream-regulatory region was amplified by PCR and used in analysis of the promoter. Two plasmids were constructed using the cbhB promoter coupled to the lacZ reporter gene with the hygromycin marker gene hph, under control of the strong gpdA promoter, in opposite orientations (Fig. 1). These constructs were used to transform A. fumigatus strain AF293. Transformant strains AF110-1 and AF110-2 from plasmid pMJB110 and strains AF201-1 and AF201-2 from plasmid pMJB201 were selected for further analysis. None of the strains showed any obvious phenotypic variation from the wild-type strain and each carried only a single copy of the transforming plasmid (data not shown).

To study the regulation of the cbhB promoter over time, β-galactosidase activity was determined in crude protein extracts from mycelia. Low levels of native β-galactosidase activity were detected in the wild-type strain AF293, reaching a maximum of around 0.9 U mg⁻¹ of protein 8 h after transfer to CMC (Fig. 3). No activity could be detected after 4 h on glucose.

For transformant strains AF110-1 and AF110-2, β-galactosidase activity significantly above basal levels could be seen after only 2 h on CMC and activity increased dramatically at the 4-h time-point (Fig. 3). In comparison, no activity could be detected in the 4-h glucose sample. The cbhB reporter construct is therefore regulated in a manner similar to the endogenous gene. Interestingly, transformants that were generated using the pMJB201 plasmid, in which the cbhB promoter is divergent from the selectable marker, showed no β-galactosidase activity above basal levels even in the presence of CMC (Fig. 2).

**mRNA levels in transformant strains**

To determine whether the absence of β-galactosidase activity in pMJB201 transformed strains (AF201-1 and -2) represented inactivity of the promoter, quantitative analysis of mRNA levels were performed using real-time PCR. Cultures were grown as for the β-galactosidase activity experiment and samples was taken 4 h after transfer to CMC or glucose.

Expression of the reporter gene could be detected in both pMJB110-transformed strains (AF110-1 and -2) and pMJB201-transformed strains (AF201-1 and -2). AF110-1 and -2 transformants on CMC showed lacZ levels that were approximately a third of the control β-tubulin level; however, expression could still be detected on glucose at values c.
100-fold lower than that on CMC (Fig. 4a). In AF201-1 and AF201-2, \( \text{lacZ} \) expression was c. 300-fold less than \( \beta \)-tubulin levels on CMC, accounting for the inability to detect \( \beta \)-galactosidase activity in these strains. On glucose, \( \text{lacZ} \) expression was c. 100-fold lower than on CMC, 30 000-fold less than the \( \beta \)-tubulin levels (Fig. 4a).

Expression of the native \( \text{cbhB} \) could not be detected in any of the transformed strains on glucose, mirroring the results from the wild-type strain (data not shown). However, on CMC expression of \( \text{cbhB} \) could be clearly seen (Fig. 4a). The levels of \( \text{cbhB} \) expression in the highly expressing \( \text{lacZ} \) strains AF110-1 and -2 were reduced c. fivefold compared with the AF201-1 and -2 strains, indicating that the activity of the reporter gene was impacting on the expression levels of the native \( \text{cbhB} \) gene (Fig. 4a).

Currently, the most efficient inducible promoter system for use in \( \text{Aspergillus} \) strains is the \( \text{A. nidulans alcA} \) expression system. We coupled the \( \text{lacZ} \) reporter cassette to the \( \text{alcA} \) promoter to compare directly the \( \text{alcA} \) and \( \text{cbhB} \) expression systems. The expression levels achieved using the \( \text{alcA} \) promoter were very high and comparable to the expression of \( \beta \)-tubulin (Fig. 4b). Upon repression, a 76-fold reduction in expression was seen. The \( \text{cbhB} \) promoter system gave greater reduction of expression, with the pMJB110 strains (AF110-1 and -2) giving a 123-fold decrease, while the pMJB201 strains (AF201-1 and -2) gave a 119-fold decrease. Furthermore, the actual levels of expression on glucose were much lower for the \( \text{cbhB} \) system, particularly pMJB201 strains.

The \( \text{cbhB} \) promoter can be used to regulate RNAi

Previous examples of regulated RNAi in \( \text{A. fumigatus} \) have shown incomplete recovery of the wild-type phenotype, presumably due to the leaky nature of the inducible promoters (Romero et al., 2003). The strong regulation exhibited by the \( \text{cbhB} \) promoter suggests that it could provide an improved regulatory element for RNAi.
An RNAi construct under the control of the cbhB promoter was produced (pALBR2; Fig. 1), targeting the alb1 gene, which encodes a polyketide synthetase involved in spore pigmentation (Tsai et al., 1998). In alb1⁻ strains, conidia have white spores as opposed to the green-blue appearance of wild-type spores. The RNAi construct was transcribed divergently from the marker gene hph. Of four transformants containing the RNAi construct, all showed a spore colour change on CMC, two (R2B and R2C) having pure white colonies indicative of complete repression of alb1 (Fig. 5a). On glucose, R2B showed no obvious spore colour difference compared with the wild-type strain, while R2C...
presented light green spores indicating partial reduction in alb1 activity.

Real-time PCR was performed on the R2B strain to assess expression of alb1 (Fig. 5b). alb1 expression on glucose matched that of the wild-type strain, indicating that no RNAi was occurring. On CMC, levels of alb1 expression were < 2% of that seen in the wild-type strain.

Discussion

We have demonstrated that the cbhB promoter of A. fumigatus is tightly regulated, providing quantitative evidence for this. Few studies have to date focused on the quantitative aspects of the control of gene expression by specific gene promoters in Aspergilli, although this is a prerequisite for the development of a robust expression system.

Our data for cbhA resemble those reported for the cbhA gene of A. nidulans, which was induced by cellulose and repressed by glucose (Lockington et al., 2002). However, the regulation of the A. nidulans homologue seems to differ in that it is negatively regulated by the global nitrogen metabolism repression system mediated by areA. In contrast, we were able to see expression of cbhA despite our experiments being carried out in Vogel’s medium, which uses ammonium nitrate as the nitrogen source. This indicates that there are differences in cbhA regulation between the two species of Aspergillus.

We found that the cbhB promoter gave very low expression levels on glucose and c. a 100-fold increase in the level of message on CMC in our reporter strains. In our hands, the A. nidulans alcA promoter yielded c. an 80-fold increase in level of induced over repressed, and showed a higher level of gene expression under repression than cbhB on glucose. The effectiveness of A. nidulans alcA in A. fumigatus has previously been studied by Romero et al. (2003). Here, the endogenous essential nudC gene was placed under the control of the alcA promoter. Transformants containing the construct were unable to grow on glucose (repressing conditions) but could grow on threonine (inducing conditions), indicating efficient control of the nudC gene. However, as the nudC gene is essential, it was not possible to determine nudC message levels under repressing conditions, although partially repressed conditions gave substantial inhibition, estimated at 3–4% of induced levels. We suggest that if a high level of control is required, the cbhB system may be more suitable than alcA. It should be noted that our studies used CMC as the sole carbon source. For comparative assessment of growth, CMC alone may not be appropriate as it is such a poor carbon source. Such studies can, however, be performed using culture media supplemented with glycerol, as this does not negatively affect the expression of the cbhB promoter (M. Bromley, unpublished observation).

Two inducible systems based on noncarbon-based induction have been reported for use in Aspergilli. The thiA promoter of A. oryzae is responsible for regulation of thiamine biosynthesis and is repressed at the transcription and translation levels by thiamine and has been shown to regulate egfp expression in A. nidulans as well as A. oryzae (Shoji et al., 2005). In addition, an E. coli promoter system based on the tetracycline-resistance operon has been adapted for use in A. fumigatus (Vogt et al., 2005). This system provides a significant advance in inducible promoter systems in filamentous fungi as it has the potential for regulation during in vivo infection experiments. However, both these systems share the same problem as the previously described carbon-based systems in that significant expression still occurs in repressed or ‘off’ states.

RNAi is an increasingly important system for the investigation of gene function in fungi; however, little has as yet been done with a regulated system in the human pathogen A. fumigatus. Mouyna et al. (2004) used the A. niger glaA promoter in A. fumigatus to drive RNAi constructs directed against alb1 and β(1-3) glucan synthase (fks1). While inhibition was successful, under repressed conditions (xylose), the fks1 construct was found to be leaky, with RNA levels 25% of those in wild-type strain. In comparison, our levels on glucose were the same as the wild type. Like us, Mouyna et al. observed a range of phenotypes on induction, from partial to full knockout. It was suggested that this could be due to different sites of integration of the RNAi construct, leading to variations in the efficiency with which the construct is expressed. This could be overcome using a nonintegrative plasmid such as AMA.

For studies of gene function and RNAi experiments in particular, effective repression is as important a factor as good levels on induction. Our data indicate that, in A. fumigatus, cbhB is more suitable than existing promoter systems and should facilitate the identification of essential genes in A. fumigatus, thereby contributing to the discovery of novel anti-fungal drug targets.

Acknowledgements

The authors would like to thank Peter Hey for his technical assistance. The cbhB promoter system is the subject of patent application GB602435.

References
