Available online at www.sciencedirect.com
SCIENCE DIRECT®
Fungal Genetics and Biology 42 (2005) 1–8
FUNGAL GENETICS AND BIOLOGY
www.elsevier.com/locate/yfgbi

Technological advancement

A rapid method for promoter exchange in *Aspergillus nidulans* using recombinant PCR

Majid Zarrin, Abigail C. Leeder, Geoffrey Turner*

Department of Molecular Biology and Biotechnology, Firth Court, University of Sheffield S10 2TN, UK

Received 16 July 2004; accepted 6 October 2004

Abstract

Recombinant PCR has been used to generate linear fragments for promoter replacement by transformation in *Aspergillus nidulans*. A cassette vector carrying the *pyr-4* non-homologous selectable marker and conditional promoter Pr-*alcA* was constructed for use as a template for PCR, and is suitable for testing the function of essential genes. Two genes involved in polar growth, *cotA* and *bemA*, were used to assess the system. Efficient targeting was possible with both genes using approximately 500 bp of flanking homologous sequence. Depending on yield, the linear PCR product could be used directly for transformation, or after first cloning into a suitable vector. *bemA*, a putative homologue of the *Saccharomyces cerevisiae BEM1* gene was identified through sequence comparison. In *A. nidulans*, this protein appears to have a similar role to the yeast Bem1p, which acts as a scaffold protein involved in the establishment of cell polarity.

Index Descriptors: Promoter replacement; Recombinant PCR; *Aspergillus nidulans*; *alcA* promoter; Polar growth; *BEM1*; Homologous integration

1. Introduction

The recent completion and automatic annotation of the genome sequence of *Aspergillus nidulans* (http://www.broad.mit.edu/annotation/fungi/aspergillus/), one of the major genetic model systems for filamentous fungi, has led to an urgent need to develop rapid techniques for analysis of gene function. Although a wide range of genetic manipulation techniques, including transformation, gene disruption and deletion, and promoter exchange have been used for many years (reviewed in Turner, 1994), many of these techniques require time consuming vector construction steps. In *Saccharomyces cerevisiae*, the development of a large number of toolkits has dramatically accelerated functional analysis, resulting in systematic analysis of the entire genome. Such tools include gene deletion, promoter exchange, epitope tagging, GFP-labelling (Baudin et al., 1993; Bellí et al., 1998; Sheff and Thom, 2004; Wach et al., 1994; Zeng et al., 2004), and the TAP-tag system for studying protein interactions (Gavin et al., 2002; Ho et al., 2002; Honey et al., 2001). Many of these tools depend on a high frequency of homologous integration at the gene locus of interest using PCR-based approaches, with convenient cassettes as templates, and short, flanking regions of homology, often provided by long oligonucleotides which can be synthesized directly to avoid any need for complex constructions. While homologous integration of transforming vectors is also used in *A. nidulans*, experience has indicated that relatively long regions of homologous sequence (1 kb or more) are required for a good efficiency of targeted integration (Turner, 1994). Krawchuk and Wahls (1999) experienced some difficulties in targetting certain genes in *Schizosaccharomyces pombe*, and overcame this by using a 2-step PCR procedure (recombinant PCR), in which long left and right flanking homologous sequences were

* Corresponding author. Fax: +44 114 272 8697.
E-mail address: g.turner@sheffield.ac.uk (G. Turner).

1087-1845/$ - see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.fgb.2004.10.002

generated prior to amplification of the cassette template, and this approach might be useful for *A. nidulans*. A second advantage of *S. cerevisiae* is that deletion of essential recessive genes can be performed in diploid strains, and sporulation then gives a 2:2 ratio of wild-type and deletion mutants. Even if the deletion is lethal, a terminal phenotype can often be observed. While deletion of essential genes can also be achieved in diploids (McGoldrick et al., 1995) or heterokaryons (Oakley et al., 1990; Osmani et al., 1988) of *A. nidulans*, recovery and characterisation of severely impaired haploid mutants is often problematic. Nevertheless, this can partly be overcome by the use of the *A. nidulans alcA* conditional promoter (McGoldrick et al., 1995; Waring et al., 1989).

The aim of this work was to examine the feasibility of using a PCR-cassette approach in *A. nidulans*, making use of the *alcA* promoter and a selectable marker in the first instance to permit analysis of essential genes.

2. Materials and methods

2.1. Strains and plasmids

Aspergillus nidulans strain G191 (*pyrG89*, *pabaA1; fwA1*, *uaY9*) was used for fungal transformation. Plasmid pMS1 carries (Johns et al., unpublished; Safaie, 2000) 4.3 kb of the *cotA* gene. Plasmid pAL4 (Waring et al., 1989) carries the *alcA* promoter of *A. nidulans* and the selectable marker *pyr-4* of *Neurospora crassa*. Plasmids were propagated in *Escherichia coli* DH5α.

2.2. Construction of plasmid pMZ1

Plasmid pAL4 was digested with *Eco*RI/*Bam*HI, the 425 bp *alcA* promoter was excised from the gel and was ligated into the *Bam*HI and *Eco*RI sites of pUC19 to give pMN18. A 1896 bp *pyr-4* gene fragment was excised as an *Eco*RI fragment from plasmid pHF11 (Mohamed et al., unpublished), and ligated into *Eco*RI-digested pMN18 to give pMZ1 (Fig. 1).

2.3. Growth media and transformation

Fungal strains were grown on complete medium (CM), minimal medium (MM), and YEPD (2% yeast extract, 0.1% peptone, and 2% glucose), supplemented when required with *p*-aminobenzoate (paba), uracil, and uridine. Media composition, culture conditions, and transformation were as described by Ballance and Turner (1985). DNA (1–5 μg) was used for transformation of protoplasts.

MM containing 100 mM threonine or 1% glycerol instead of glucose was used to induce or permit expression of the *alcA* promoter, and YEPD was utilised to obtain maximum repression of Pr-*alcA*. Threonine (100 mM) and 100 mM glycerol were used together to induce Pr-*alcA* for the phenotype characterisation of *bemA* mutants. Transformant colonies were purified by streaking to single colonies four times on MM with 1% glycerol. Genomic DNA was isolated using a Wizard Genomic DNA Purification Kit (Promega). Restriction enzyme digests were performed in accordance with manufacturer's instructions (Promega, New England Biolab, and Roche). Probes for Southern blot hybridisation analysis were labelled with [^{32}P]dCTP using the Amersham Megaprime DNA labelling System.

2.4. PCR amplifications

The cassette-based method for generating a fusion PCR product for transformation and promoter replacement was adapted from Krawchuk and Wahls (1999).

All reactions were performed in a Perkin–Elmer DNA Thermal Cycler using the Expand Long Template

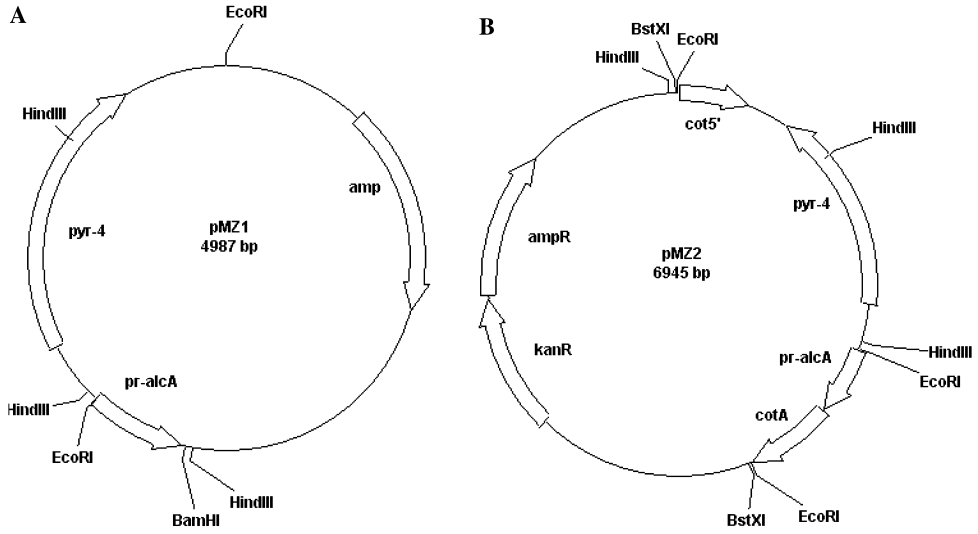

Fig. 1. Plasmids used for promoter exchange. (A) pMZ1 and (B) pMZ2.

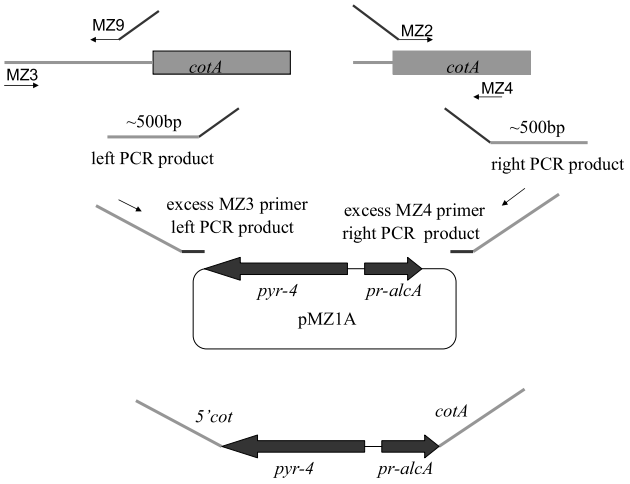

Fig. 2. Recombinant PCR for promoter exchange with *cotA*.

PCR system (Roche) or TripleMaster PCR System (Eppendorf). The oligonucleotide primers were synthesized by MWG-Biotech (Ebersberg, Germany). PCRs were done in 100 µl using 0.5 ml thin-walled PCR tubes, and overlayered with 50 µl mineral oil. The amplification of the gene targeting fragment was achieved by two-step PCR. In the first round of PCR, two fragments (left and right) of the target gene region were separately amplified. For each reaction, one of the primer pair was complementary to both the target region and the expression cassette of pMZ1. By using the left and right PCR products from the first round PCR with plasmid pMZ1 as a template for second round PCR, together with two external primers, a recombinant fragment for transformation and promoter replacement was obtained. The strategy of this method is shown in Fig. 2, and the primers used are in Table 1.

Table 1
PCR primers

Gene	Oligonucleotide sequence 5′–3′
For the amplification of ~500 bp flanking sequences of cotA	
(A) First round	
Left	
MZ3	TCGTAGCACAGTGTCGTGA
MZ9	GGCCAAGGGTTTCTCTAAGGATT
	TGGGGAGCAAGCGACAACGATTC
Right	
MZ2	CTTCCAATCCTATCACCTCGCCTC
	AAA**ATG**GACCCCAACAACAATCG
MZ4	GGTAGCGTTGAAGTTCTTCG
(B) Second round	
MZ3	TCGTAGCACAGTGTCGTGA
MZ4	GGTAGCGTTGAAGTTCTTCG
For the amplification of flanking regions of bemA	
(A) First round	
Left	
MZ16	GTCTTTTGTTGCCTGTCC
MZ14	GGCCAAGGGTTTCTCTAAGGATT
	TGGGGGTGCGACTGATAAGACT
Right	
MZ15	CTTCCAATCCTATCACCTCGCCTC
	AAATTCC**ATG**ATGGGCAAAGC
MZ17	GTCGCACTGACATCCTCT
(B) Second round (nested primers)	
MZ18	CTATCTCCTCCTTCATCC
MZ19	GAGCCAGTCGTCTCGATT

Underlined sequences in primers MZ9, MZ14 are complementary to *pyr4*. Underlined sequences in primers MZ2 and MZ15 are complementary to *alcA*. The translation start codons used for *cotA* and *bemA* are shown in bold.

2.5. Recombinant PCR for cotA promoter replacement

For first-round PCR with primers MZ3 and MZ9, MZ2, and MZ4, each reaction mixture contained 200 µM of each dNTP, 10 µl PCR buffer with 22.5 mM $MgCl_2$, 100 pmol of each primer, 2.5 U DNA Pol Mix (Roche) (*Taq* polymerase and proof-reading enzyme), and 15 ng plasmid pMS1. Hot start amplification was initiated with 5 min at 95 °C denaturation, followed by 30 amplification cycles (95 °C for 1 min, 54 °C for 1 min, and 68 °C for 2 min). PCR products from each reaction were gel-purified using a QIAGEN QIAquick kit and used in subsequent amplification.

Second-round PCR mixture was as for the first-round, except that 10 ng each of left and right PCR product from the first round of PCR, together with 200 pmol of MZ3 and MZ4, were used as primers, and 20 ng pMZ1 as template. PCR conditions were as for the first-round, except that the extension time at 68 °C was 4 min. To increase the yield of DNA the cassette was subcloned into pCR 2.1 using the Invitrogen Original TA Cloning Kit to give plasmid pMZ2 (Fig. 1), and can be excised as required with *Bst*XI.

2.6. Recombinant PCR for bemA promoter replacement

First-round PCR was performed as for *cotA*, using primers MZ16 and MZ14, MZ15, and MZ17 and an annealing temperature of 50 °C, with 300 ng *A. nidulans* genomic DNA as a template. For second-round amplification, nested primers MZ18 and MZ19 were used, $MgCl_2$ was increased to 2.75 mM, and 1 ng of each left and right PCR product from the first-round of PCR were used with 16 ng of pMZ1. Amplification was with 3.5 U TripleMaster Enzyme Mix (Eppendorf).

2.7. Homology searches

Blast searches were performed for *A. nidulans* using the partial Cereon database (http://microbial.cereon. com/), and later the Whitehead database (http://www.broad.mit.edu/annotation/fungi/aspergillus/index.html), and for *A. fumigatus* at TIGR (http://www.tigr.org/tdb/e2k1/afu1/).

2.8. Microscopy

A spore suspension of $1–5 \times 10^4$ spores in 25 ml supplemented liquid MM or YEPD was added to a sterile glass Petri dish lined with coverslips. The strain was incubated at 37 °C for the times indicated, during which conidia had germinated and adhered to the coverslips. Cells were examined with a fluorescence microscope (model DMLB; Leica). Digital images were captured by a CCD camera (model RTE; Princeton Instruments) controlled by an Apple Macintosh G4 computer running Open Lab software version 2.2.5 (Improvision). Images were exported as TIFF files and edited in Jasc Paint Shop Pro version 7.04.

3. Results

3.1. Promoter exchange with cotA

Plasmid pMZ1 (Fig. 1) was constructed to provide a cassette template containing the widely used *N. crassa pyr-4* gene as a non-homologous selectable marker (Ballance et al., 1985), and the *A. nidulans alcA* promoter, which permits controlled of expression of any gene of interest over a large dynamic range (Adams et al., 1988; Kennedy and Turner, 1996; Waring et al., 1989). To generate sufficient flanking sequence homologous to the target gene region, the method described by Krawchuk and Wahls (1999) for *S. pombe* was adopted, and has been referred to as recombinant PCR or splicing by overlap extension. To test the method, we first applied it to the cloned *cotA* gene (GenBank Accession No. AY620243), since previous work had shown an easily recognisable compact growth and hyperbranching phenotype when this gene was placed under the control of Pr-*alcA* by transformation with circular and linear plasmids (Johns et al., unpublished; Safaie, 2000). The method is illustrated in Fig. 2, and resulted in the deletion of 230 bp of sequence 5′ to the *cotA* translation start. Flanking regions were amplified in the first-round of PCR using outer primers MZ3 and MZ4, and inner primers MZ9 and MZ2 (Table 1; Fig. 3A) which included extension sequences that were complementary to the ends of the *pyr-4-alcA* cassette. In a second PCR, the flanking homologous regions (410 bp 5′ of *cotA*, and the first 513 bp of the *cotA* coding region) were joined to the *pyr-4-alcA* cassette such that *cotA* coding region was joined to the *alcA* promoter (Fig. 3B). The final product (3039 bp) was used to transform *A. nidulans*, either directly, or after cloning into pCR 2.1 to increase the yield of DNA. Pyr^+ transformants of G191 were selected on threonine (*alcA* induced), and tested on YEPD (*alcA* repressed). Using 1 μg gel purified fragment, only five transformants were initially obtained, and two of these showed the *cotA* growth phenotype on the repressing YEPD medium. To increase the yield of DNA the cassette was subcloned into pCR 2.1 to give pMZ2 (Fig. 1). The pCR 2.1 vector has *Bst*XI sites either side of the cloning site which remove the product insert from the vector, leaving some residual vector nucleotides at each end. *A. nidulans* strain G191 was transformed with 5 μg of the *Bst*XI fragment. Of the 84 transformants obtained, 56% showed the *cotA* phenotype on YEPD. Six colonies with the *cotA* phenotype were analysed by Southern blot (Fig. 4). Five showed the band pattern predicted for a single copy homologous integration, and one contained an additional unpredicted band, possibly a result of an ectopic copy of the transforming DNA.

3.2. Promoter exchange with bemA

To test the method on a gene not previously studied, a putative homologue (*bemA*) of the *BEM1* gene of *S.*

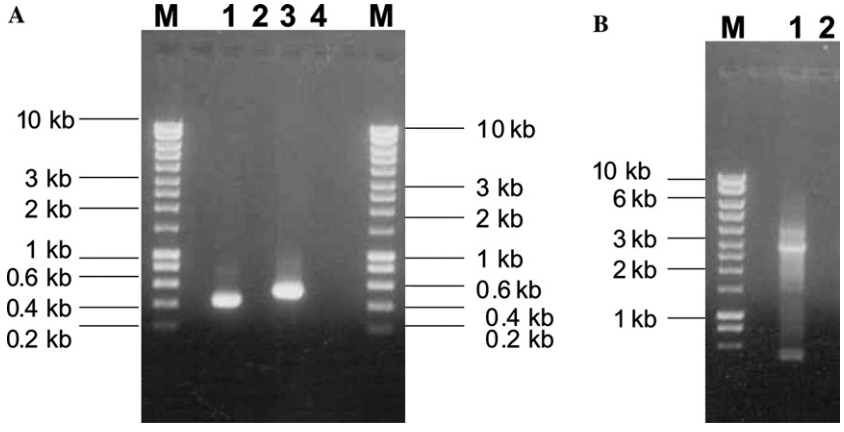

Fig. 3. PCR products of the *cotA* gene. (A) PCR amplification of two flanking regions of the *cotA* gene: lane M, hyperLadder; lane 1, a 410 bp upstream fragment of the *cotA* gene (left) using primers MZ3 and MZ9; lane 2, no template control; lane 3, a 513 bp start fragment of *cotA* gene (right) using primers MZ2 and MZ4; and lane 4, no template control. (B) The second round PCR: lane M, hyperLadder; lane 1, PCR product using primers MZ3 and MZ4; and lane 2, no template control.

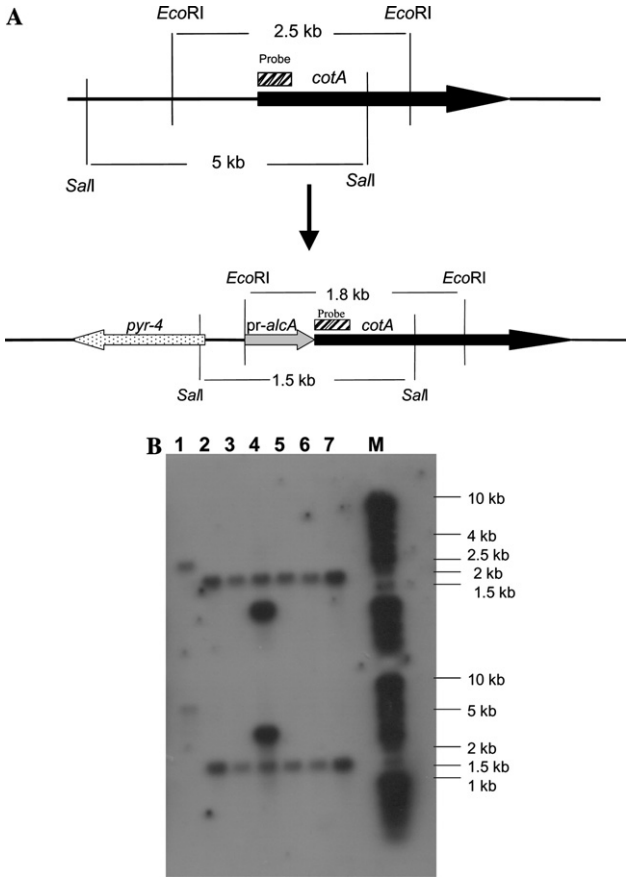

Fig. 4. Analysis of *cotA* mutants. The predicted gene replacement event and relevant restriction enduclease sites are shown in (A) (not to scale). Genomic DNA prepared from six transformants and G191 (wt) was digested with *Eco*RI and *Sal*I, and probed with 500 bp right-flanking region of *cotA* gene (B). Top: lane M, hyperLadder; lane 1, G191/*Eco*RI; lane 2, TPA7/*Eco*RI; lane 3, TPA13/*Eco*RI; lane 4, TPA18/*Eco*RI; lane 5, TPA31/*Eco*RI; and lane 6, TPA32/*Eco*RI, TPA47/*Eco*RI. Bottom: lane 1, G191/*Sal*I; lane 2, TPA7/*Sal*I; lane 3, TPA13/*Sal*I; lane 4, TPA18/*Sal*I; lane 5, TPA31/*Sal*I; lane 6, TPA32/*Sal*I; and lane 7, TPA47/*Sal*I.

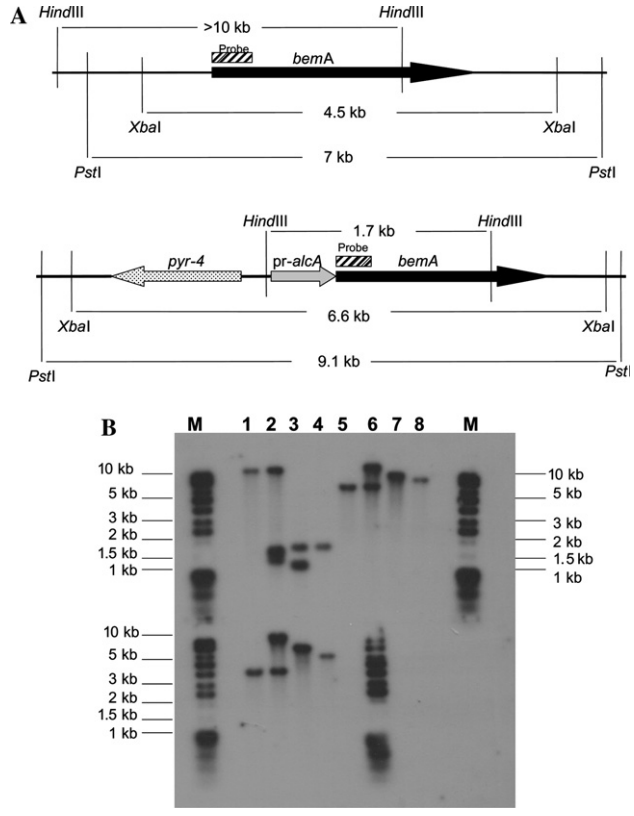

Fig. 5. Analysis of *bemA* mutants. The predicted gene replacement event and relevant restriction sites are shown in (A) (not to scale). Genomic DNA prepared from three transformants and G191 (wt) was digested with *Hin*dIII, *Pst*I, and *Xba*I, probed with 400 bp right-flanking region of *bemA* gene. (B) Top: lane M, hyperLadder; lane 1, G191/*Hin*dIII; lane2, BDA13/*Hin*dIII; lane 3, BDA24/*Hin*dIII; lane 4, BDA28/*Hin*dIII; lane 5, G191/*Pst*I; lane 6, BDA13/*Pst*I; lane 7, BDA24/*Pst*I; and lane 8, BDA28/*Pst*I. Bottom: lane M, hyperLadder; lane 1, G191/*Xba*I; lane 2, BDA13/*Xba*I; lane 3, BDA24/*Xba*I; and lane 4, BDA28/*Xba*I.

cerevisiae, which is involved in polar growth, was identified from the Cereon (3X) genome sequence data. Though the partial sequence obtained by Cereon did not provide 5′ region upstream of the coding region, it has been observed that the first SH3 domain of *BEM1* is not essential for function in yeast (Matsui et al., 1996). Recombinant PCR was therefore used to place the *alcA* promoter 5′ of an internal ATG (methionine 159 in the now complete Whitehead sequence of feature AN7030.2, GenBank Accession No. XM411167.). Since no product was obtained in the second round PCR, external primers MZ16 and MZ17 were replaced with nested primers MZ18 and MZ19 (Table 1). After purification, the final PCR product was re-amplified with MZ18 and MZ19 to obtain sufficient DNA for direct transformation, incorporating flanking homologous sequences of 365 and 345 bp.

Direct transformation of G191 with 5 μg of the PCR product yielded 89 transformants, 47% of which were unable to grow beyond the germling stage on YEPD but were indistinguishable from the wild-type on threonine medium (Fig. 6). To confirm that putative *bemA* mutants which responded to carbon source control, resulted from homologous integration, transformants were analysed by Southern blot hybridisation (Fig. 5). BDA28 showed a band pattern consistent with single copy integration at the *bemA* locus. BDA13, whose growth morphology did not respond to repression of *alcA*, retained the band pattern expected for the wild-type *bemA* locus together with additional bands suggesting ectopic integration of the transforming DNA. Though BDA24 had lost the wild-type band pattern, and responded to *alcA* repression, the observed bands did not correspond to those predicted for a straightforward gene replacement, suggesting some rearrangement at the *bemA* locus.

3.3. Phenotype of alcA–bemA strain

Homologous integrant BDA28 was tested on various media to analyse the phenotype. When grown on induc-

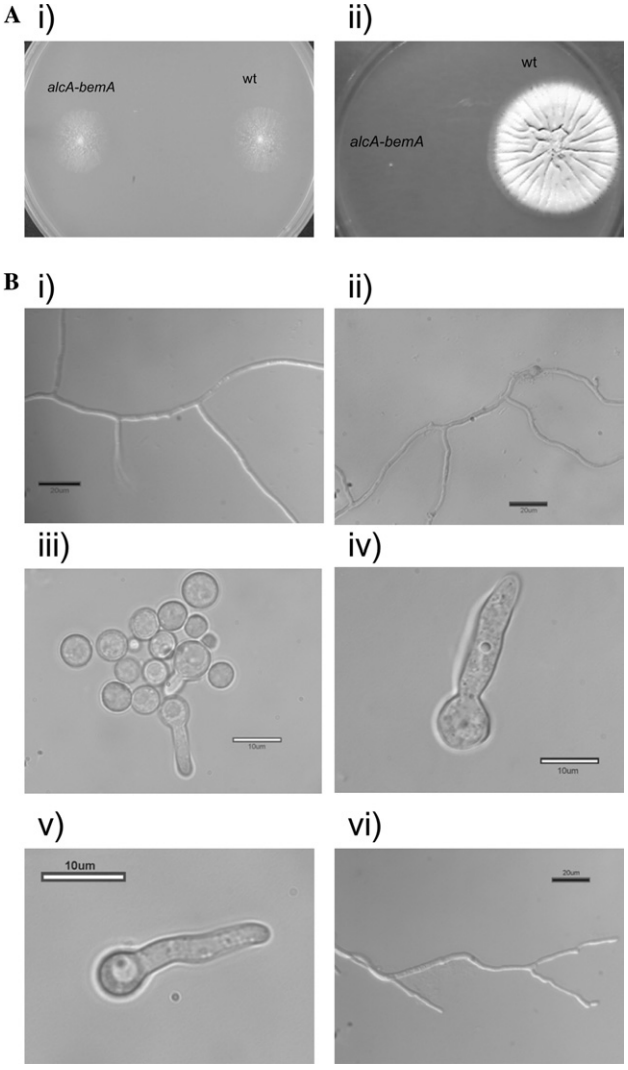

Fig. 6. A comparison between *bemA*$^{+}$ (G191) and *alcA–bemA* (BDA28) strains on inducing and repressing media. (A) Growth on agar plates, left colony BDA28, right colony G191. (i) AMM containing 100 mM threonine at 37 °C for 48 h. (ii) YEPD at 37 °C for 72 h. (B) Early germlings. (i) *bemA*$^{+}$ and (ii) *alcA–bemA* strain on MM plus threonine and glycerol for 15 h at 37 °C. (iii) *alcA–bemA* strain on YEPD for 15 h and (iv) for 36 h. (v) *alcA–bemA* strain grown on MM with 1% glucose for 15 h and (vi) 36 h. Scale bars 20 μm (i), (ii), and (vi) or 10 μm (iii)–(v).

ing media, the strain exhibited a phenotype similar to the wild-type (Figs. 6A(i) and B(i)(ii)). There was no delay in germination or in the production of lateral branches and development continued as normal. Since the transforming DNA has inserted between the first and second SH3 domains of *bemA*, this suggests that the first SH3 domain of the protein may not be essential for function, as no obvious defect is seen.

Most of the spores were unable to germinate when grown for 22 h on strongly repressing media (YEPD, Fig. 6B(iii)). The cells were very swollen and the largest extensions that any developed were short in length with a wide diameter. Even after 36 h incubation, only a minority had germinated and these only to a limited extent (Fig. 6B(iv)). A small proportion of the cells had swollen to the point that they had lysed.

On MM containing 1% glucose, conidiospores were swollen and many are un-germinated (Fig. 6B(v)). However, several spores were able to produce a small germ tube and after 36 h a minority of these had developed a second germ tube and small lateral branches (Fig. 6B(vi)).

Table 2
Frequency of germ-tube emergence for wild-type and *alcA–bemA* spores

	% of spores germinated			
	15 h	18 h	22 h	36 h
Wild-type	92.2	92.1	90.8	95.3
alcA–bemA Inducing conditions (glycerol plus threonine)	86.5	88.9	89.2	91.1
alcA–bemA Repressing conditions (YEPD)	15.7	14.6	17.7	29.5

Ungerminated—Polar growth was absent. Spores spherical.
Germinated—Polar growth was evident. Projection observed.
% values are the mean of three experiments (100 spores per experiment).

3.4. Quantitative counts of germ tube emergence

To assess the frequency of germ tube emergence a sample of 100 spores was observed after incubation at 37 °C for varying time periods. Any spores that were seen to have localized directional growth were scored as having a germ tube, regardless of its length. This was repeated in triplicate for each condition and an average taken (Table 2).

With the *alcA* promoter induced, cells were seen to germinate at a level comparable to wild-type. After 15 h incubation at 37 °C approximately 87% of the spores had produced a germ tube, compared to 92% of wild-type spores (Table 2). The small difference seen can be accounted for by the faster growth rate of the wild-type cells. In contrast, less than 18% of the spores had germinated on YEPD after 22 h. This proportion increased to almost 30% after 36 h of incubation, but was still significantly lower than wild-type spores. The majority of spores on YEPD failed to establish any germ tube, and in those which did, growth ceased immediately after the initial germination event. None produced a secondary germ tube or lateral branches.

4. Discussion

The recombinant PCR approach to gene manipulation offers the possibility of accelerating studies on gene function in *A. nidulans* to exploit the genome sequence data. The method is rapid because it does not involve complex vector construction which is a time consuming process, though in some cases, it may be necessary to

clone the final PCR product into a suitable vector in order to obtain sufficient DNA for fungal transformation. We also found that nested primers can help given improved yields in the second round of PCR.

Though there are no systematic studies on the length of homologous sequence required for efficient targeting (>50%) in *A. nidulans*, researchers have generally used >1 kb in circular or linear vectors. In order to minimise the length of the final PCR product, we used approximately 500 bp flanking sequences, and still observed an acceptable frequency of targeting with the two genes used in this study. Since targeting efficiency seems to be locus dependent (G. Turner, unpublished observations), it is probably safer to use at least 500 bp, and this method can easily be adapted to increase the length of flanking homology.

Genes in the *A. nidulans* database are predicted and annotated automatically, and it is inevitable that there will be some errors in predicted translation start sites. Any large scale functional analysis will have to take account of this, and the start sites could be confirmed following determination of the transcription start site by 5′ RACE, for example. While specific deletion of the putative endogenous promoter region is desirable, it may not be straightforward if adjacent genes in close proximity are divergently transcribed.

The *bemA* gene, judged to be a putative homologue of the *S. cerevisiae BEM1* gene by BLAST searching, seems to be required for polar growth in *A. nidulans*. The *BEM1* gene of yeast encodes a protein with two SH3 domains, and mutants are defective in cell polarisation (Chenevert et al., 1992). Bem1p is a scaffold protein which interacts with other proteins involved in establisment of polar growth, and it has been shown that deletion of the first SH3 domain has no observable effect on gene function (Matsui et al., 1996). The *alcA* promoter exchange that we carried out on *bemA*, designed before the full genome sequence was available, resulted in separation of the first SH3 domain, still controlled by the endogenous *bemA* promoter, from the truncated Pr-alcA-bemA by the *pyr-4* marker, but no differences can be seen between the wild-type strain and the *alcA-bemA* strain grown on media which induce *alcA*. This suggests that the first SH3 domain may be unnecessary for polar growth in *A. nidulans*, as is the case in yeast. On the other hand, maximum downregulation of *alcA* on YEPD medium leads to loss of polarity establishment in most conidia, and no significant growth over a period of 3 days. This effect is more severe than in yeast, where deletion of *BEM1* leads to a moderate growth defect and abnormal morphology.

While not all genes required for polar growth in filamentous fungi may be recognisable as yeast homologues (Gatherar et al., 2004), the detailed understanding of polar growth in yeast (Casamayor and Snyder, 2002; Pruyne and Bretscher, 2000a,b) offers the opportunity of investigating the role of putative homologues in filamentous fungi (Harris and Momany, 2004), and the approach described offers a rapid method to do this.

Note added in proof

Similar PCR-based methods have recently been described in two independent publications: Yu et al. (Fungal Genet. Biol. 41, 973–981); Yang et al. (Eukaryot. Cell 3, 1359–1362).

Acknowledgments

This work was supported by the Iranian Ministry of Health and Medical Education (M.Z.) and the Biotechnology and Biological Sciences Research Council (A.L. and G.T.).

References

Adams, T.H., Boylan, M.T., Timberlake, W.E., 1988. *brlA* is necessary and sufficient to direct conidiophore development in *Aspergillus nidulans*. Cell 54, 353–362.

Ballance, D.J., Turner, G., 1985. Development of a high frequency transforming vector for *Aspergillus nidulans*. Gene 36, 321–331.

Baudin, A., Ozier-Kalogeropoulos, O., Denouel, A., Lacroute, F., Cullin, C., 1993. A simple and efficient method for direct gene deletion in *Saccharomyces cervisiae*. Nucleic Acids Res. 21, 3329–3330.

Bellí, G., Garí, E., Aldea, M., Herrero, E., 1998. Functional analysis of yeast essential genes using a promoter–substitution cassette and the tetracycline-regulatable dual expression system. Yeast 14, 1127–1138.

Casamayor, A., Snyder, M., 2002. Bud-site selection and cell polarity in budding yeast. Curr. Opin. Microbiol. 5, 179–186.

Chenevert, J., Corrado, K., Bender, A., Pringle, J., Herskowitz, I., 1992. A yeast gene (BEM1) necessary for cell polarization whose product contains SH3 domains. Nature 356, 77–79.

Gatherar, I.M., Pollerman, S., Dunn-Coleman, N., Turner, G., 2004. Identification of a novel gene *hbrB* required for polarised growth in *Aspergillus nidulans*. Fungal Genet. Biol. 41, 463–471.

Gavin, A., Bösche, M., Krause, R., et al., 2002. Functional organization of the yeast proteome by systematic analysis of protein complexes. Nature 415, 141–147.

Harris, S.D., Momany, M., 2004. Polarity in filamentous fungi: moving beyond the yeast paradigm. Fungal Genet. Biol. 41, 391–400.

Ho, Y., Gruhler, A., Heilbut, A., et al., 2002. Systematic identification of protein complexes in *Saccharomyces cerevisiae* by mass spectrometry. Nature 415, 180–183.

Honey, S., Scheinder, B.L., Schieltz, D.M., Yates, J.R., Futcher, B., 2001. A novel multiple affinity purification tag and its use in identification of proteins associated with a cyclin-CDK complex. Nucleic Acids Res. Vol. 29, E24.

Kennedy, J., Turner, G., 1996. δ-(L-α-aminoadipyl)-L-cysteinyl-D-valine synthetase is a rate limiting enzyme for penicillin production in *Aspergillus nidulans*. Mol. Gen. Genet. 253, 189–197.

Krawchuk, M.D., Wahls, W.P., 1999. High-efficiency gene targeting in *Schizosaccharomyces pombe* using a modular, PCR-based approach with long tracts of flanking homology. Yeast 15, 1419–1427.

McGoldrick, C.A., Gruver, C., May, G.S., 1995. *myoA* of *Aspergillus nidulans* encodes an essential myosin-I required for secretion and polarized growth. J. Cell Biol. 128, 577–587.

Matsui, Y., Matsui, R., Akada, R., Toh-e, A., 1996. Yeast *src* homology region 3 domain-binding proteins involved in bud formation. J. Cell Biol. 133, 865–878.

Oakley, B.R., Oakley, C.E., Yoon, Y., Jung, M.K., 1990. Gamma-Tubulin is a component of the spindle pole body that is essential for microtubule function in *Aspergillus nidulans*. Cell 61, 1289–1301.

Osmani, S.A., Engle, D.B., Doonan, J.H., Morris, N.R., 1988. Spindle formation and chromatin condensation in cells blocked at interphase by mutation of a negative cell cycle control gene. Cell 52, 241–251.

Pruyne, D., Bretscher, A., 2000a. Polarization of cell growth in yeast. I. Establishment and maintenance of polarity states. J. Cell Sci. 113, 571–585.

Pruyne, D., Bretscher, A., 2000b. Polarization of cell growth in yeast. II. The role of the cortical actin cytoskeleton. J. Cell Sci. 113, 365–375.

Safaie, M., 2000. Genetic control of hyphal cell growth and polarity in *Aspergillus nidulans*. PhD Thesis. University of Sheffield.

Sheff, M.A., Thom, K.S., 2004. Optimized cassettes for fluorescent protein tagging in *Saccharomyces cerevisiae*. Yeast 21, 661–670.

Turner, G., 1994. Vectors for gene manipulation. In: Martinelli, S.D., Kinghorn, J.R. (Eds.), *Aspergillus*: 50year on, Progress in Industrial Microbiology, 29. Elsevier Science, Amsterdam, pp. 641–665.

Wach, A., Brachat, A., Pöhlmann, R., Philippsen, P., 1994. New heterologous modules for classical or PCR-based gene disruptions in *Saccharomyces cerevisiae*. Yeast 10, 1793–1808.

Waring, R.B., May, G.S., Morris, N.R., 1989. Characterization of an inducible expression system in *Aspergillus nidulans* using alcA and tubulin-coding genes. Gene 79, 119–130.

Zeng, G.F., Pypaert, M., Slayman, C.L., 2004. Epitope tagging of the yeast K(+) carrier Trk2p demonstrates folding that is consistent with a channel-like structure. J. Biol. Chem. 279, 3003–3013.