The *chsA* gene from *Aspergillus nidulans* is necessary for maximal conidiation

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Abstract

A fragment from the open reading frame of the cloned *chsA* gene from *Aspergillus nidulans* was deleted and replaced with the *argB* gene. The resulting construct was used to replace the wild-type *chsA* gene in an *argB* deletion strain. The growth and morphology of the vegetative hyphae from the resulting *chsA* disruptant strain were indistinguishable from those of a wild-type strain but the chitin content of the hyphae from the disruptant was reduced to approximately 90% of that of wild-type. The disruptant showed reduced ability to produce the asexual spores (conidia) that are formed by differentiated aerial hyphae called conidiophores. The ability to form undifferentiated aerial hyphae was not impaired in the disruptant. The conidiophores and conidia produced by the disruptant were indistinguishable from those of wild-type. Conidium formation by the disruptant grown on a variety of media was reduced to about 30% of the wild-type. A *chsE* null strain did not show a defect in conidiation but a strain in which both *chsA* and *chsE* were inactivated produced about 3% of the conidia of wild-type. That finding supports the hypothesis that *chsA* and *chsE* encode a partially redundant function necessary for conidiophore development. © 2000 Published by Elsevier Science B.V. All rights reserved.

Keywords: Chitin; Chitin synthase; *chsE* gene; Redundant function

1. Introduction

Chitin is a linear polysaccharide of N-acetylglucosamine residues linked by β-1,4 glycosidic linkages. The polymer plays an important structural role in the cell walls of all true fungi [1,2]. Studies in the yeast *Saccharomyces cerevisiae* have shown that the synthesis of the polysaccharide is complex, requiring three related genes that encode chitin synthase isozymes [3]. Chitin synthase genes form a divergent family that can be divided into five classes [4,5]. The C-termini from the enzymes are relatively conserved and are thought to encode the catalytic domain [6]. The N-termini of the isozymes are more highly divergent and may contain sequences necessary for the correct localization and regulation of the enzymes. The three chitin synthase isozymes from *S. cerevisiae* are functionally distinct since each synthesizes a unique chitin subfraction at specific sites in the yeast cell at specific times in the cell cycle [3]. In the developmentally more complex filamentous fungi, chitin synthesis is even more intricate and involves additional chitin synthase genes. Five chitin synthase genes have been identified in the filamentous ascomycete *Aspergillus nidulans* [5,7–11]. Seven chitin synthase genes have been identified in *Aspergillus fumigatus* [7,12].

The phenotypes of *A. nidulans* mutants carrying inactivated chitin synthase genes indicate that the isozymes encoded by *chsB*, *chsD* and *chsE* are each responsible for the synthesis of a functionally distinct chitin subfraction [5,7,10,11,13]. The *chsB* gene is responsible for the synthesis of a minor chitin subfraction in hyphae that are necessary for the normal growth, morphology and organization of hyphal cells [7,11]. The *chsD* gene [5], also referred to as *csmA* [8,13], is responsible for the synthesis of a chitin subfraction that is necessary for the structural rigidity of hyphal walls and of conidiophore vesicles. Inactivation of the *chsE* gene [5], also referred to as *chsD* [10], results in strains that lack about 30% of hyphal chitin but are morphologically and developmentally normal.

Strains carrying an inactivated copy of either the *A. nidulans chsA* or *chsC* genes [9–11] are reported to exhibit no morphological or developmental defects. A possible explanation for these results is that the isozymes en-
coded by the genes are functionally redundant with iso-
yzymes encoded by other chitin synthase genes. Motoyama
et al. [10] showed that a strain in which both \chsA and
\chsE (referred to as \chsD in that paper) had been inacti-
vated produced about 7% of the conidia of a wild-type
strain. In this paper, we report that inactivation of \chsA
alone in fact leads to a defect in conidiophore formation
and to a deficit in the chitin content of vegetative hyphae.

2. Materials and methods

2.1. Strains, media and culture conditions

The following strains were used in this study: PB 001
(yA2 pabaA1; veA1); RMS 010 (biA1 methG1;
\argB::trpC\DeltaB; trpC801 veA1); RMS 011 (yA2 pabaA1;
\argB::trpCAB; trpC801 veA1); PB 122 (yA2 pabaA1;
\chsE::argB; \argB::trpCAB; trpC801 veA1); PB 142 (yA2 pabaA1;
\chsA::argB; \argB::trpCAB; trpC801 veA1) and PB 146 (yA2 pabaA1;
\chsA::argB; \chsE::argB; \argB::trpCAB; trpC801 veA1). Strains were grown at 37°C on YG me-
dium (0.5% yeast extract, 1% glucose) or on the minimal
medium described by Clutterbuck [14]. PB 141 was crossed
with \textit{RMS 010} to produce PB 142. PB 142 was crossed
with PB 122 to give PB 146.

2.2. DNA manipulations

A cosmids containing the entire \chsA gene was identified
from an ordered cosmids library in vector pc-
sAX [15] using PCR and the primers GCGTCTAGATA-
CCGAGACTCGTACGAAGA and GCCCTCATAGTG-
ATCTCAGATACAGGAG. The cosmid was digested
with SaI and XbaI and a 3.9-kb fragment containing
the entire open reading frame was cloned into the SaI-
XbaI site of Bluescript KS" to yield plasmid pYM4.
pYM4 was cut with \textit{ClaI} and \textit{PstI} to remove a 2-kb in-
ternal coding fragment from the \chsA gene and a 1.7-kb
\textit{ClaI}-\textit{PstI} fragment from plasmid pRP8 that contained the
entire \argB gene from \textit{A. nidulans} was ligated into the site,
yielding pYM5. Strain RMS 011 was transformed with
pYM5 that had been digested with \textit{KpnI}. Transformations
were performed according to Yelton et al. [16].

2.3. Conidiation

Strains were inoculated at the center point of solid me-
dium contained in 90-mm Petri dishes. The plates were in-
cubated at 37°C until the colonies had reached a diameter
of 80 mm. Each dish was flooded with 10 ml of 50% glycerol and the conidia were harvested by scraping the
surface of colonies with a nichrome wire scraper. For each
strain, the conidia from three plates were pooled and the
number of conidia present in the suspension was counted
in a hemocytometer. Following dilution of the suspensions,
the number of viable conidia from each strain was
determined by colony count on YG medium supplemented
with 0.6 M KCl.

2.4. Chitin content

The chitin content of hyphae was assayed as previously
described [5]. The data presented represent the means for
five independent experiments. In each experiment, three
cultures were grown for each strain and the mycelia
from each culture were pooled for determination of amino
sugar and protein content.

3. Results

Strain RMS 011 was transformed with plasmid pYM5
that had been linearized and \arg"' transformants were
selected. Fig. 1A shows a restriction map of the \chsA
region of the wild-type \textit{A. nidulans} genome and that pre-
dicted for a transformant produced by homologous inte-
gration of the fragment. We screened transformants for
individuals with a Southern blot pattern consistent with
the disruption event. Several transformants showed the
blot pattern predicted for a disruption (Fig. 1B). PB 141
was chosen for characterization in this study.

Wild-type \textit{A. nidulans} strains form copious numbers of
brightly colored asexual spores (conidia) that are produced
by differentiated aerial hyphae known as conidiophores.
On YG-based media, PB 141, a yellow spored strain con-
taining the disrupted \chsA gene, could be visually distin-
guished from an isogenic wild-type strain, PB 001, since
the yellow color of the mutant colony was not as intense
as that of PB 001 (Fig. 2). Microscopic examination of the
colony surface of PB 141 grown on YG showed numerous
aerial hyphae but a decreased number of conidiophores
compared to the wild-type strain. The conidiophores and
conidia produced by PB 141 were indistinguishable from
the wild-type (not shown).

We compared the number of conidia produced by
\chsA::argB and \chsA::argB, \chsE::argB strains with a
wild-type grown on a variety of media (Table 1). On the
four media, the \chsA::argB disruptant strain produced an
average of 31% of the conidia of the wild-type strain. The
\chsA::argB, \chsE::argB strain produced an average of
3.1% of the conidia of wild-type. The viability of the con-
idia from the wild-type and mutant strains produced on all
media was comparable and averaged about 80% of the
microscopic count (data not shown). The reduced viable
count was due to the fact that conidia frequently occur in
chains that could be distinguished in the hemocytometer
but give rise to single colonies in the viable count experi-
ments.

The chitin content of hyphae of the \chsA::argB strain

350

Fig. 1. A: The upper map shows a partial restriction map of genomic DNA from the region of the chsA gene. The lower map shows the map predicted following homologous integration of the transforming DNA into the chsA region. B: Southern blots of wild-type (PB 001) genomic DNA (lane 1) and DNA from PB 141 (lane 2) digested with SspI and probed with the SalI-XbaI fragment containing the chsA gene. The bar represents 1 kb.

Fig. 2. Colonies of PB 001 (top row) and PB 141 (bottom row) grown 4 days at 37°C on: YG medium (left) and YG medium+0.6 M KCl (right).
Table 1
Spore formation by a wild-type and chitin synthase null mutants

<table>
<thead>
<tr>
<th>Strain</th>
<th>Relevant genotype</th>
<th>Medium</th>
<th>YG</th>
<th>YG+0.6 M KCl</th>
<th>Minimal</th>
<th>Minimal+0.6 M KCl</th>
</tr>
</thead>
<tbody>
<tr>
<td>PB 001</td>
<td>Wild-type</td>
<td></td>
<td>3.2×10^6 (100%)</td>
<td>3.9×10^6 (100%)</td>
<td>2.7×10^6 (100%)</td>
<td>1.8×10^6 (100%)</td>
</tr>
<tr>
<td>PB 141</td>
<td>ΔchsA::argB</td>
<td></td>
<td>1.2×10^6 (38%)</td>
<td>1.2×10^6 (31%)</td>
<td>7.0×10^5 (26%)</td>
<td>5.6×10^5 (31%)</td>
</tr>
<tr>
<td>PB 122</td>
<td>ΔchsE::argB</td>
<td></td>
<td>3.3×10^6 (103%)</td>
<td>3.4×10^6 (87.2%)</td>
<td>2.8×10^6 (104%)</td>
<td>2.0×10^6 (111%)</td>
</tr>
<tr>
<td>PB 146</td>
<td>ΔchsA::argB, ΔchsE::argB</td>
<td></td>
<td>8.9×10^6 (2.8%)</td>
<td>1.4×10^6 (3.6%)</td>
<td>7.1×10^5 (2.6%)</td>
<td>5.7×10^5 (3.2%)</td>
</tr>
</tbody>
</table>

Data are presented as the number of conidia per ml. The percentages of conidia produced by the wild-type control are given in parentheses.

(PB 141) was compared with that of a wild-type strain (PB 001). Five independent experiments were performed in which each strain was grown and processed for chitin content. In each experiment, the chitin content of the mutant was lower than that of the control strain. The average chitin content of PB 001 in the five experiments was 1.20 (± 0.16) mg N-acetylglucosamine equivalents mg⁻¹ protein while PB 141 averaged 1.05 (± 0.09) mg N-acetylglucosamine equivalents mg⁻¹ protein. Using the paired t-test for the five experiments, the mean difference in chitin content (0.15 mg N-acetylglucosamine equivalents mg⁻¹ protein) was statistically significant with a probability of 0.0082.

4. Discussion

Previous work has shown that inactivation of the chsB, chsD or chsE genes leads to strains with distinguishable phenotypic defects [5,7,11,13]. These observations indicate that each gene is responsible for the synthesis of a unique chitin subfraction that serves a distinct organizational, structural or developmental role. The data presented in this paper demonstrate that inactivation of chsA results in no defect in conidiation. The disruption strategies used in the two studies. In our work, a substantial fraction of the open reading frame of chsA was replaced with the argB gene while in the previous study, a simple insertion of a disrupting marker was made into the 3′ region of chsA. In the latter case, it is possible that the gene retained partial activity.

Strains carrying an inactivated copy of chsA can be distinguished phenotypically from chsB and chsD disruptants and consequently chsA is functionally distinct from chsB and chsD. The hyphal chitin deficiency and the defect in conidiation of chsA disruptants might be related. The hyphal chitin synthesized by the chsA-encoded enzyme could be necessary for the initiation of conidiophore formation. Alternatively, the enzyme might also synthesize chitin in conidiophores and the lack of that chitin is responsible for the conidiation defect in chsA disruptants.

The chsA disruptants are only partially blocked in conidium formation (30% of the conidia of wild-type are produced) and the conidiophores and conidia that are produced have the wild-type morphology. Inactivation of chsE does not result in a defect in conidiation (this study, [5,10]). Strains carrying inactivated copies of both chsA and chsE have a severe block in conidiation, producing only 3–7% of the conidia of a wild-type strain (this study, [10]). These observations are consistent with the proposal that the chsA and chsE-encoded enzymes are capable of synthesizing the same chitin subfraction. The reduction in conidiation caused by inactivation of chsA and the finding that chsE disruptants conidiate at wild-type levels could be explained by assuming that, in wild-type strains, the chsA-encoded activity is present in excess while the chsE-encoded activity is present in limiting amounts. The fact that double disruptants are capable of 3–7% of the conidiation of wild-type suggests that chitin synthases encoded by other genes may be capable of fulfilling the chsA/chsE function in conidiation to a limited extent.

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References


