# GENETICS AND PHYSIOLOGY OF RESPIRATION IN *ASPERGILLUS NIDULANS*

A THESIS PRESENTED IN PARTIAL FULFILMENT OF THE
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**REBECCA ELIZABETH GARDINER**

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# ABSTRACT

Obligate aerobes such as *Aspergillus nidulans* primarily use the classical respiratory pathway for ATP production. However, the use of alternative energy-producing processes in *A. nidulans* was first speculated upon when cytochrome *c*-deficient strains were observed to be viable upon fermentable carbon sources. It was therefore postulated that the *cycA*$^-$ strains of *A. nidulans* may use fermentation and an alternative respiratory pathway to compensate for the non-functioning cytochrome *c*-dependent pathway.

Characterisation of the *A. nidulans* cytochrome *c*-deficient strains was carried out. The growth parameters for strain A68 were consistent with other cytochrome *c* mutants; the strain grew more slowly than the corresponding wildtype strain on fermentable carbon sources, and produced higher levels of ethanol. Spectral analysis confirmed the lack of detectable levels of cytochrome *c* in the *cycA*$^-$ strains, and decreased levels of cytochrome *c* oxidase, consistent with the non-functioning cytochrome *c*-dependent respiratory pathway. The presence of a hemoglobin-like molecule in the *cycA*$^-$ and *cycA*$^+$ strains was determined by CO binding assays.

Inhibitor studies determined the presence of an alternative respiratory pathway in *cycA*$^-$ and *cycA*$^+$ strains of *A. nidulans*. An active cytochrome *c*-dependent pathway was found to be present in the *cycA*$^+$ strains, yet absent from the *cycA*$^-$ strains. Results also suggested the presence of a putative third terminal oxidase in the *cycA*$^-$ and *cycA*$^+$ strains. Increased levels of *b*-type heme observed in the redox spectra of the *cycA*$^-$ strains were suggested to be associated with the putative third terminal oxidase.

Therefore, the *cycA*$^-$ strains of *A. nidulans* appear to use fermentation and the alternative respiratory pathway to compensate for the non-functioning cytochrome *c*-dependent pathway. The putative hemoglobin molecule identified in these strains may also function as a terminal oxidase, in addition to the putative third terminal oxidase.

PCR amplification with degenerate primers was carried out to confirm the presence of an *AOX* gene in *cycA*$^+$ and *cycA*$^-$ strains of *A. nidulans*. The product of the *AOX* gene is likely to function as a terminal oxidase in the alternative pathway. A comparison of fungal and plant AOX protein sequences was carried out. A conserved cysteine residue which has

been implicated in dimer formation and pyruvate regulation was found to be absent from the fungal sequences.

A preliminary expression study of the *A. nidulans AOX* gene was carried out by RT-PCR. The putative regulatory elements identified within the *A. nidulans AOX* gene promoter are also located within the promoters of other respiratory-related genes (eg. *A. nidulans cyc*A) and genes involved in reducing the formation of reactive oxygen species (ROS) (eg. *A. nidulans catA-C* and *sod1*). This implies similar mechanisms of regulation, which may be controlled in a coordinated manner.

A functional analysis of the *A. nidulans cyc*A gene promoter was carried out to identify important regulatory elements. Reporter constructs containing *cyc*A-*lacZ* fusion genes were transformed into *A. nidulans*. Although integrated at the *arg*B locus, the constructs had very low levels of *lacZ* expression (with the exception of the positive control). A number of parameters were investigated, and a 'promoter switch' experiment commenced, however the cause of the faulty *cyc*A-*lacZ* expression system was not determined.

Functional expression of the *A. nidulans cyc*A gene promoter in yeast was also attempted, as regulatory mechanisms controlling cytochrome *c* expression are believed to be analogous in *A. nidulans* and *Saccharomyces cerevisiae*. Reporter constructs containing *cyc*A-*lacZ* fusion genes were transformed into *S. cerevisiae*, with resulting low β-galactosidase activity, similar to the results in *A. nidulans*. The wildtype strain of *A. nidulans* from which the *cyc*A gene promoter fragment was amplified was shown by spectral analysis to have low levels of cytochrome *c*, in comparison to other wildtype strains of *A. nidulans*. Therefore, it is possible that the low levels of *lacZ* expression from the *cyc*A-*lacZ* fusion genes may be representative of the level of *cyc*A expression in that strain. However, low levels of *lacZ* expression were also observed for the *S. cerevisiae* positive control, indicating that the expression system was not working properly. Therefore, the expression of the *A. nidulans cyc*A gene promoter in *S. cerevisiae* could not be assessed in this study.

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# ABBREVIATIONS

| | |
|---|---|
| ANCF | *Aspergillus nidulans* CCAAT binding factor |
| AOX | alternative oxidase |
| ATP | adenosine triphosphate |
| bp | base pair |
| cAMP | cyclic adenosine monophosphate |
| CCR | carbon catabolite repression |
| CCHL | cytochrome *c* heme lyase |
| CO | carbon monoxide |
| COX | cytochrome *c* oxidase |
| *CycA* | *A. nidulans* gene encoding cytochrome *c* |
| *CYC1* | *Saccharomyces cerevisiae* gene encoding iso-1-cytochrome *c* |
| *CYC7* | *S. cerevisiae* gene encoding iso-2-cytochrome *c* |
| DNA | deoxyribonucleic acid |
| EMP | Embden-Meyerhof-Parnas pathway |
| ETC | electron transport chain |
| HAP1 | *S. cerevisiae* heme activated protein, mediating oxygen regulation |
| HAP2/3/4/5 | *S. cerevisiae* CCAAT binding factor, mediating carbon regulation |
| HMC | high molecular weight complex |
| hr | hour(s) |
| Kb | kilobase pair(s) |
| KCN | potassium cyanide |
| MAI | maximum amount of inhibition |
| min | minute(s) |
| NADH | nicotinamide adenine dinucleotide |
| ORF | open reading frame |
| PCR | polymerase chain reaction |
| RPM | repression modules |
| RNA | ribonucleic acid |
| ROS | reactive oxygen species |
| RT-PCR | reverse transcribed PCR |
| SHAM | salicyl hydroxamic acid |
| SOD | Superoxide dismutase |
| TCA | tricarboxylic acid cycle |
| *tsp* | transcriptional start site |

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