REGULATION OF THE *ASPERGILLUS NIDULANS* CYTOCHROME *C* GENE

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ABSTRACT

The filamentous fungus *Aspergillus nidulans* has been genetically and biochemically well-characterised and thus provides an attractive model for studies on the regulation of eukaroytic gene expression. This study was undertaken to investigate the factors affecting respiratory function in *A. nidulans*. Due to the central role of cytochrome *c* in oxidative respiration, this study was focused on the cytochrome *c* gene and primarily upon how oxygen availability affects its expression.

The *Aspergillus nidulans* cytochrome *c* gene (*cycA*) appears to be transcriptionally activated in response to oxygen availability (Raitt *et al.*, 1994). In the yeast *S. cerevisiae*, oxygen availability activates its cytochrome *c* genes via a heme-activated protein HAP1, which binds to the promoter region of each gene (Pfiefer *et al.*, 1989). Since heme is only synthesised in the presence of oxygen, activity of the HAP1 protein serves as an intracellular signal of oxygen availability.

In the upstream region of the *cycA* gene, a sequence with homology to the *S. cerevisiae* HAP1 binding site was present (Raitt, 1992). To determine the significance of the putative HAP1 binding site and the role of other promoter sequences in the *A. nidulans cycA* gene, a promoter-reporter vector was constructed. However, upon sequencing of the *cycA* promoter in the reporter vector, a sequencing error was discovered in the published *cycA* gene by Raitt *et al.* (1994) which affected the position of the major translational start site. Further examination of the *cycA* sequence also revealed a possible undetected intron (Intron I). To determine the number of introns in the *cycA* gene, RT-PCR was performed on *cycA* RNA. Sequencing of the RT-PCR amplified products showed that the previously undetected intron (Intron I) was present, and that the *cycA* gene contains three instead of two introns as published by Raitt *et al.* (1994). Since the published ATG start site was located within Intron I, a new translational start site was proposed. The major consequences of these changes to the *cycA* gene was that the putative HAP1 site was now located within the coding region of the gene, and therefore could not be a regulatory element. In addition only 247 bp of *cycA* promoter sequence remained cloned for analysis. To obtain additional promoter sequence, an *A. nidulans* genomic library was screened with a *Bam*HI cDNA probe containing 224 bp of the 5' region of the *cycA* gene. Three positive clones were obtained, of which λLM9 and λLM5 were identical, and λLM19 was an overlapping clone with λLM9 and λLM5. Restriction enzyme and Southern blott analysis of the two overlapping *cycA* clones, revealed that 2.1 kb *Eco*RI fragments from both clones contained the 5' region. The 2.1 kb *Eco*RI fragment from λLM9 was cloned into pUC18 and sequenced. The completed

upstream sequence of the *A. nidulans* cytochrome *c* gene was obtained, and putative regulatory signals including the HAP1 binding site were found, and compared with published promoter sequences which regulate the expression of respiratory-encoding genes from yeast.

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