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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 eukaryotic 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 *BamHI* 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 *EcoRI* fragments from both clones contained the 5' region. The 2.1 kb *EcoRI* 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 HAPI binding site were found, and compared with published promoter sequences which regulate the expression of respiratory-encoding genes from yeast.
ACKNOWLEDGEMENTS

Firstly, I would like to thank my supervisor Rosie Bradshaw, for keeping faith in me, and for her continual enthusiasm and encouragement, even when my thesis was not going according to plan! Her support was much appreciated.

I would also like to thank all past and present members of the Microbiology and Genetics Department. Thanks everyone for your friendship, you guys made it a great place to work in. In particular, I would like to thank our neighbouring lab, Scott base, who we were always borrowing things from, especially Carolyn Young, who somehow found time out of her busy research to give all of us MGU students loads of time saving advice, Mike Collett for answering all my questions so extensively and patiently, and Austen Ganley for being around to listen and to entertain us out at the farm. This also applies to Kate and Mike Collett who we shared many yummy dinners and good times with.

Many thanks goes to everyone from my lab, Dianne-for all her Aspergillus related advice, Paul-for providing lots of laughs, Carmel-thanks for being such a great friend, Anita-for her lively personality which helped keep us sane, Branwen-for your support especially during those hard times, Brendon-for all those friendly chats, and Bek-for technical support, I hope you have more luck with this project than I did! Also thanks to Tania who was always popping into our lab with lots of smiles.

A big thank you must also go to my family for their love and support. I would also like to thank my special friend Rowena. Thanks for putting up with me, especially during our four years flatting together, and for all your strength and encouragement, which went along way towards the accomplishment of this thesis.

Lastly, I have Richard to thank for many many things, I hardly know how to adequately express my gratitude and love. Thank you Rich for giving me your support, love, encouragement, guidance, cooking more than I did, and all those sacrifices you made because I was too busy! Also I must mention that your scientific advice was invaluable. I am very lucky to have you for a future husband!
TABLE OF CONTENTS

ABSTRACT.................................................................................................................. ii

ACKNOWLEDGEMENTS............................................................................................... iv

TABLE OF CONTENTS................................................................................................. v

LIST OF TABLES........................................................................................................... xi

LIST OF FIGURES......................................................................................................... xii

CHAPTER 1. INTRODUCTION...................................................................................... 1

1.1 THE ROLE OF THE CYTOCHROME C PROTEIN............................................. 1

1.2 WHY STUDY THE ASPERGILLUS NIDULANS CYTOCHROME C GENE?........... 1

1.3 REGULATION OF THE SACCHAROMYCES CEREVISIAE CYTOCHROME C GENES........................................................................................................... 2

1.3.1 HAP1 Regulation of CYC1 and CYC7 in Response to Oxygen Availability .... 3

1.3.1.1 Structure and Function of the HAP1 Protein............................................. 3

1.3.1.2 Multi-sequence Recognition by HAP1..................................................... 4

1.3.2 Regulation of CYC1 and CYC7 in Response to Carbon Source by the Yeast CCAAT-binding Factor ................................................................. 8

1.3.2.1 The Yeast CCAAT-binding Factor (HAP2/3/4/5 Complex).................. 8

1.3.3 Other factors affecting CYC7 expression......................................................... 10

1.4 REGULATION OF THE KLUYVEROMYCES LACTIS CYTOCHROME C GENE (KICYC1) ........................................................................................................... 10

1.5 REGULATION OF THE ASPERGILLUS NIDULANS CYTOCHROME C GENE (cycA)................................................................................................................. 12

1.4.1 Regulation of the cycA gene in response to Environmental Parameters........... 12

1.4.2 Analysis of the 5' Region of the cycA gene.................................................... 13
1.4.2.1 Putative HAP1 Binding Site ................................................. 13
1.4.2.2 Putative Upstream Opening Reading Frame (uORF) ........................ 13

1.5 AIMS AND OBJECTIVES ................................................................ 13

CHAPTER 2. MATERIALS AND METHODS ........................................ 17

2.1 FUNGAL AND BACTERIAL STRAINS, LAMBDATA CLONES
AND PLASMIDS ............................................................................ 17

2.2 MEDIA ...................................................................................... 17
2.2.1 Luria Broth ............................................................................ 17
2.2.3 MYG Media ........................................................................... 17
2.2.4 SOC Medium .......................................................................... 17
2.2.5 NZCYM .................................................................................. 17
2.2.6 TB Top Agarose ...................................................................... 17

2.3 COMMON BUFFERS AND SOLUTIONS ....................................... 17
2.3.1 1 x TAE Buffer ....................................................................... 19
2.3.2 1 x TBE Buffer ....................................................................... 19
2.3.3 TE Buffer (10/1) ..................................................................... 19
2.3.4 TE Buffer (10/0.1) .................................................................. 19
2.3.5 10 x Sequencing TBE Buffer .................................................... 19
2.3.6 TES (10/1/100) ..................................................................... 19
2.3.7 STET Buffer ........................................................................... 19
2.3.8 SM Buffer: ............................................................................. 19
2.3.9 Hybridisation Solution ............................................................ 19
2.3.10 50 x Denhardt's Solution ....................................................... 19
2.3.11 Tris-Equilibrated Phenol ......................................................... 19
2.3.12 20 x SSC .............................................................................. 19
2.3.13 10 x Gel Loading Buffer ....................................................... 19
2.3.14 Standard DIG Buffer ........................................................... 19
2.3.15 DIG Buffer 1 ......................................................................... 20
2.3.16 DIG Buffer 2 ......................................................................... 20
2.3.17 DIG Buffer 3 ......................................................................... 20
2.3.18 Blocking Reagent stock solution ............................................ 20
2.3.19 Alkaline Lysis Solution I .......................................................... 20
2.3.20 Alkaline Lysis Solution II ....................................................... 20
2.3.21 Alkaline Lysis Solution III .............................................................. 20
2.4 GROWTH AND MAINTENANCE OF CULTURES ........................................ 20
2.5 DNA PREPARATIONS ............................................................................. 20
2.5.1 Rapid Boiling Plasmid Preparation ...................................................... 20
2.5.2 Small Scale Alkaline Lysis Plasmid Preparation .................................... 20
2.5.3 Large Scale Alkaline Lysis Plasmid Preparation .................................... 21
2.5.4 Purification of Plasmid DNA by Cesium Chloride-Ethidium Bromide
Gradient ........................................................................................................ 22
2.5.5 Preparation of DNA from Fungal Cultures ........................................... 22
2.5.6 Preparation of Lambda Phage DNA by Liquid Lysate .............................. 23
2.5.7 Purification of DNA by Phenol-Chloroform Extraction and Ethanol
Precipitation ..................................................................................................... 23
2.6 STANDARD PROCEDURES .................................................................... 24
2.6.1 Restriction Enzyme Digests ................................................................... 24
2.6.2 Agarose-gel Electrophoresis of DNA ..................................................... 24
2.6.3 Determination of Molecular Weights ..................................................... 24
2.6.4 Determination of DNA Concentration ................................................... 25
2.6.4.1 Quantification of DNA Concentration by Electrophoresis .................... 25
2.6.4.2 Spectrophotometric Determination of DNA Concentration and
Purity ................................................................................................................. 25
2.7 AMPLIFICATION OF DNA BY THE POLYMERASE CHAIN REACTION
(PCR) ............................................................................................................... 25
2.8 DNA CLONING PROCEDURES ............................................................... 26
2.8.1 Gel Purification of DNA ....................................................................... 28
2.8.2 CAP Treatment of DNA ....................................................................... 28
2.8.3 Ligation Reactions ................................................................................. 28
2.8.4 Transformation of Escherichia coli ......................................................... 29
2.8.4.1 Preparation of Electroporation Competent E. coli Cells ....................... 29
2.8.4.2 Electroporation of E. coli ................................................................... 29
2.8.4.3 Preparation of CaCl2-Competent Cells .............................................. 30
2.8.4.4 CaCl2 Transformation of E. coli ............................................................ 30
2.9 COLONY HYBRIDISATION USING THE DIG DETECTION SYSTEM ......... 30
2.9.1 Colony Lifts ........................................................................................ 30
2.9.2 Colony Hybridisation ............................................................................ 31
2.9.3 Preparation of DIG-Labelled Probe ...................................................... 31
2.9.4 Detection of Hybridised DIG-labelled Probe ........................................... 32
2.10 DNA SEQUENCING .................................................................................... 32
2.10.1 Sequenase Version 2.0 Sequencing Protocol .......................................... 32
2.10.2 Amplicycle Sequencing Kit Protocol .................................................... 33
2.11 RNA PROCEDURES .................................................................................. 33
2.11.1 Precautions Taken for Working With RNA .......................................... 33
2.11.2 Agarose-gel Electrophoresis of RNA .................................................. 34
2.11.3 Determination of Fungal RNA Concentration and Purity .................... 34
2.12 RNA PREPARATION .................................................................................. 34
2.12.1 Aspergillus nidulans Spore Suspension Preparation ............................. 34
2.12.2 Isolation of RNA from Aspergillus nidulans ........................................ 35
2.13 RT-PCR ANALYSIS OF RNA .................................................................... 35
2.13.1 Reverse Transcriptase Synthesis of cDNA ........................................... 35
2.13.2 Amplification of cDNA by PCR ............................................................ 36
2.14 SOUTHERN BLOTTING AND HYBRIDISATION ....................................... 36
2.14.1 Southern Blotting ................................................................................... 36
2.14.3 Southern Blot Hybridisation ................................................................. 37
2.14.4 Stripping Probes from Southern Blots .................................................. 38
2.15 LIBRARY SCREENING BY PLAQUE HYBRIDISATION ............................. 38
2.15.1 Plating Phage lambda ............................................................................ 38
2.15.2 Plaque Lifts ............................................................................................ 39
2.15.3 Hybridisation of lambda Phage DNA to [alpha-32P]dCTP-Labelled Probe 39

CHAPTER 3. CONSTRUCTION OF PROMOTER-REPORTER VECTORS AND DISCOVERY OF A SEQUENCING ERROR .................................. 40

3.1 INTRODUCTION ........................................................................................... 40
3.2 CLONING STRATEGY .................................................................................. 40
3.2.1 The lacZ Reporter Vector ...................................................................... 40
3.2.2 PCR Amplification of the cycA Promoter Fragments .............................. 40
3.2.3 General Preparation of pAN923-42B Vector and 0.68 kb cycA Promoter 43
3.3 LIGATIONS AND TRANSFORMATIONS OF THE 0.68 kb cycA PROMOTER ............................................. 46
3.3.1 Ligation of 0.68 kb promoter directly into pAN923-42B .......................................................... 46
3.3.2 Ligation of 0.68 kb Promoter into T-Tailed pUC18 Vector ......................................................... 53
3.3.3 Ligation of 0.68 kb Promoter into Commercially CAP-treated pUC18 ........................................... 55
3.3.4 Ligation of 0.68 kb promoter into pAN923-42B ................................................................. 55

3.4 LIGATIONS AND TRANSFORMATIONS OF THE cycA Δ HAP1 PROMOTER ........................................... 58

3.5 SEQUENCING OF THE 0.68 kb PROMOTER IN PLASMID R122 ........................................................... 58

CHAPTER 3 DISCUSSION ...................................................................................................................... 64

3.6 PROBLEMS WITH CLONING INTO pAN923-42B VECTOR ............................................................. 64

3.7 IMPLICATIONS FROM THE DISCOVERY OF THE SEQUENCING ERROR IN THE PUBLISHED cycA GENE BY RAITT et al (1994) .......................................................... 64

CHAPTER 4. RT-PCR ANALYSIS OF cycA RNA ..................................................................................... 69

4.1 INTRODUCTION .............................................................................................................................. 69

4.2 DEVELOPMENT OF A RT-PCR STRATEGY .................................................................................... 69

4.3 RT-PCR RESULTS .......................................................................................................................... 75
4.3.1 Sequencing of RT-PCR Products .................................................................................................. 78

CHAPTER 4 DISCUSSION ...................................................................................................................... 83

4.4 THE cycA GENE CONTAINS A THIRD INTRON AND HAS A CORRECTED N-TERMINAL REGION ........ 83

4.5 ALL THREE INTRONS OF THE cycA GENE ARE LOCATED AT CONSERVED POSITIONS ................. 86

CHAPTER 5. CLONING AND ANALYSIS OF THE 5' REGION OF THE cycA GENE .......................... 89

5.1 INTRODUCTION ............................................................................................................................. 89

5.2 SCREENING OF AN A. NIDULANS LIBRARY ................................................................................. 89
5.3 SOUTHERN BLOTTING AND RESTRICTION MAPPING OF POSITIVE
λ CLONES

5.4 SEQUENCE ANALYSIS OF CLONED λ DNA SHOWN TO HYBRIDISE
TO THE cycA GENE

5.4.1 Subcloning and Sequencing of the 2.1 kb Fragment

5.4.2 Confirmation that the 2.1 kb Fragment Corresponds to Regions of the
A. nidulans A18 Genome

5.4.3 Analysis of the cycA promoter sequence

5.4.3.1 Putative HAP1 UAS

5.4.3.2 Putative HAP2/3/4/5 (Yeast CCAAT-binding Factor) UAS

5.4.3.3 Putative CREA Binding Site

5.4.3.4 Other Regulatory Elements

CHAPTER 5 DISCUSSION

5.5 TATA BOX

5.6 THE ANCF (A. nidulans CCAAT BINDING FACTOR) COMPLEX
MAY REGULATE THE CYCA GENE

5.7 THE A. nidulans CYCA GENE MAY BE REGULATED BY A HAPI-LIKE PROTEIN

CHAPTER 6. SUMMARY AND CONCLUSIONS

APPENDICES

Appendix 1.0 Vector Maps

Appendix 2.0 Sequence Data

REFERENCES
LIST OF TABLES

Table 2.1. Strains, λ clones and plasmids ..................................................... 18

Table 2.2. PCR and Sequencing Primers .......................................................... 27

Table 3.1. Transformations A, B & C (with pAN923-42B vector) ......................... 47

Table 3.2. Expected Restriction Digestion Fragment Profiles for Ligation of 0.68 kb Promoter into pAN923-42B ................................................................. 49

Table 3.3. Transformation D (with pAN923-42B vector) .................................... 52

Table 3.4. Expected Restriction Enzyme Profiles for Ligation of 0.68 kb Promoter into Plasmid pUC18 ................................................................. 54

Table 3.5. Transformations E & F (with CAP-treated pUC18 vector) ..................... 56

Table 3.6. Transformation G (with pAN923-42B vector) .................................... 57

Table 4.1. PCR Products Expected From RT-PCR Analysis ................................ 74

Table 5.1. Restriction Enzyme Digestions of λ clones ........................................ 95
LIST OF FIGURES

Figure 1.1. Projection of HAP1 binding at CYC7 and CYC1 onto DNA helices (Pfiefer et al., 1987) .................................................................................................................. 6

Figure 1.2. Comparison of HAP1 binding sites in S. cerevisiae and A. nidulans .... 14

Figure 3.1. The lacZ reporter vector pAN923 .................................................................. 41

Figure 3.2. PCR primer binding sites within the cycA promoter fragments .............. 44

Figure 3.3. Restriction enzyme analysis of plasmids pAN923, R122 and R120 ....... 50

Figure 3.4. Comparison of the cycA nucleotide sequence from plasmid R122 with the published cycA sequence (Raitt, 1992) ......................................................... 60

Figure 3.5. Autoradiograph of sequence obtained from the cycA gene cloned into plasmid  R41 ............................................................ 60

Figure 3.6. The published nucleotide sequence of the cycA gene (Raitt et al., 1994) .................................................................................................................. 62

Figure 3.7. How the sequencing error affects the cycA amino-terminal sequence..... 66

Figure 4.1. Re-estimation of the cycA transcription start point ............................... 70

Figure 4.2. RT-PCR outline showing the relative positions of primers .................. 72

Figure 4.3. RT-PCR analysis of total RNA from A. nidulans strain A18 ............... 76

Figure 4.4. cDNA sequence of the 596 bp RT-PCR product ................................. 79

Figure 4.5. The corrected nucleotide sequence of the cycA gene ......................... 81

Figure 4.6. Alignment of cytochrome c N-terminal protein sequences ............... 84

Figure 4.7. Alignment of three cytochrome c protein sequences showing intron positions .................................................................................. 87

Figure 5.1a. Restriction digestion profiles of λLM9 and λLM19 ............................ 91

Figure 5.1b. Southern Blot of λLM9 and λLM19 ...................................................... 91

Figure 5.2. Restriction maps of λLM9 and λLM19 isolated from an A. nidulans genomic library that hybridised to the cycA gene ................................. 93
Figure 5.3. Nucleotide sequencing strategy for the 5' region of the cycA gene............ 96
Figure 5.4. Nucleotide sequence of the 5' region of the cycA gene.................... 98
Figure 5.5a. Restriction digestions of A. nidulans A18 genomic DNA................... 101
Figure 5.5b. Southern blot of A. nidulans A18 genomic DNA............................ 101
Figure 5.6. Comparison of known yeast and putative A. nidulans HAP1 UASs........ 103