Redox regulation of an AP-1-like transcription factor, YapA, in the fungal symbiont *Epichloë festucae*

A thesis presented in partial fulfilment of the requirements for the degree of

Doctor of Philosophy (PhD) in

Genetics

at Massey University, Manawatu,

New Zealand.

Gemma Maree Cartwright

2013

Abstract

Reactive oxygen species (ROS) are emerging as important regulators required for the successful establishment and maintenance of the mutualistic association between the fungal endophyte *Epichloë festucae* and its grass host *Lolium perenne* (perennial ryegrass). The generation of reactive oxygen species (ROS) by the fungal NADPH oxidase, NoxA, has previously been shown to regulate hyphal growth of *E. festucae in planta*, a result that has led to the hypothesis that fungal-produced ROS are key second messengers in the symbiosis. However, the highly reactive nature of these molecules dictates that cells possess efficient redox sensing mechanisms to maintain ROS homeostasis and prevent oxidative damage to cellular components such as DNA, lipids and proteins. The *Saccharomyces cerevisiae* Gpx3-Yap1 and *Schizosaccharomyces pombe* Tpx1-Pap1, two-component H_2O_2 sensors, serve as model redox relays for coordinating the cellular response to ROS. While proteins related to the Yap1 and Pap1 basic-leucine zipper (bZIP) transcription factors have been identified in a number of filamentous fungi, the components involved in the upstream regulation remain unclear. This thesis presents an investigation into the role of the *E. festucae* Yap1 homologue, YapA, and putative upstream activators GpxC and TpxA, homologues of Gpx3 and Tpx1, respectively, in responding to ROS. YapA is involved in responding to ROS generated at the wound site following inoculation into ryegrass seedlings. However, deletion of *yapA* did not impair fungal colonisation of the host, indicating functional redundancy in systems used by *E. festucae* to sense and respond to plant-produced ROS. In culture, deletion of *E. festucae yapA* renders the mutants sensitive to only a subset of ROS and this sensitivity is influenced by the stage of fungal development. In contrast to the H_2O_2-sensitive phenotype widely reported for fungi lacking the Yap1-like protein, the *E. festucae yapA* mutant maintains wild-type mycelial resistance to H_2O_2 but conidia of the *yapA* mutant are sensitive to H_2O_2. Using a degron-tagged GFP-CL1 as a reporter, we found YapA is required for the expression of the spore-specific catalase, *catA*. Moreover, YapA is activated by H_2O_2, through disulfide bond formation, independently of both GpxC and TpxA, suggesting a novel mechanism of regulation exists in *E. festucae*. This work provides a comprehensive analysis of the role and regulation of the AP-1 transcription factor pathway in a filamentous fungal species

Acknowledgements

In many ways a PhD is like running a marathon. At the beginning the task seems huge and the finish line an unimaginable distance away. With sheer determination and perseverance and most importantly a great support crew, the road to a doctoral degree becomes a challenging and rewarding journey.

Firstly thank you to my supervisor Prof. Barry Scott, who was there every step of the way, guiding and supporting. I am in awe of your depth of knowledge and seemingly limitless enthusiasm for my project. Thank you also to my co-supervisor Dr. Rosie Bradshaw for your encouragement and advice.

Ruth, you were the whole package. Your impeccable organisation and guidance got me off to a flying start in the lab. With your friendship and support I was able to enjoy, rather than endure, my PhD years. Arvina, thank you for keeping everything in the lab running so smoothly and always with a smile on your face.

To the post-docs in Scottbase lab, past and present, Yvonne, Tetsuya, Sarah, Carla, Conni, Matt and Sanjay. Thank you for always having time to answer my questions, discuss results and share the occasional moment of mirth. Sarah Brown, I must single you out for imparting the wisest words of all "It'll be fine". To the rest of you docs, pre-docs, under-docs and maybe someday-docs in the lab, Emma, Milena, Daniel, Will, Rola, Alex, Pip, Chris, Leonie and Yonathan. Thank you for the engaging discussions, friendship and fun. Thank you for always eating the cupcakes so dutifully, without complaint, even when the cupcake influx reached an all-time high in the later stages of the PhD.

To Doug, Jianyu and Jordan, at the MMIC (my second home). Thank you not only for your technical assistance and advice, but also for all the good times and laughter shared. To Ann, Cynthia, Katrina, Tara and Paul, thank you for keeping everything running like a well-oiled machine. To Dr. Andrew Sutherland-Smith, who assisted with the protein modelling and Dr. Murray Cox who assisted with the statistical analysis. Thank you for allowing me to explore subject areas I hadn't encountered since my undergraduate papers. To Carlene Starck, thank you for your mentoring and counseling. Talking to someone who had 'been there, done that' helped to get me through.

Thanks to the many of you who took the time to review and comment on this work. Your input has been invaluable.

To my non-science friends and running buddies, especially Andrena, thank you for keeping the balance.

To my Mum and Dad, I am incredibly thankful for all of the love, support and encouragement you have given me throughout the years. I want you to realise just how important you have been in getting me to where I am today.

To Iain, my rock, my role model, my number one. Thank you for being so awesome.

This research was supported by a Top Achiever Doctoral Scholarship from the Tertiary Education Commission and funding from the Lincoln Bio-Protection Centre of Research Excellence (CoRE).

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Abbreviations

aa	Amino acid
Aa	*Alternaria alternata*
Amp	Ampicillin
Amp^R	Ampicillin resistant
Ao	*Aspergillus oryzae*
Bc	*Botrytis cinerea*
BLAST	Basic local alignment search tool
BLASTn	Nucleotide database search using a nucleotide query
BLASTp	Protein database search using a protein query
bp	Base pair(s)
C	Cysteine
Ca	*Candida albicans*
c-CRD	C-terminal cysteine-rich domain
cDNA	Complementary DNA
CDS	Coding DNA sequence
CFW	Calcofluor white
Ch	*Cochliobolus heterostrophus*
CIAP	Calf intestinal phosphatase
C_P	Peroxidatic cysteine
Cp	*Claviceps purpurea*
C_R	Resolving cysteine
CRD	Cysteine-rich domain
Cys	Cysteine
DAPI	4′,6-diamidino-2-phenylindole
dATP	Deoxyadenine triphosphate
DEPC	Diethylpyrocarbonate
DIC	Differential interference contrast

DIG	Digoxigenin
DNA	Deoxyribonucleic acid
dNTP	Deoxynucleotide triphosphate
dpi	Days post inoculation
DsRed	Discosoma red fluorescent protein
EDTA	Ethylene diamine tetra-acetic acid
EGFP	Enhanced green fluorescent protein
EMSA	Electrophoretic mobility shift assays
Fg	*Fusarium graminearum*
FGI	Fungal Genome Initiative
Fo	*Fusarium oxysporum*
FS	Flanking sequence
g	Gram
gDNA	Genomic DNA
Gen^R	Geneticin resistant
GFP	Green fluorescent protein
GPx	Glutathione peroxidase
Grx	Glutaredoxin
GSH	Glutathione
GST	Glutathione S-transferase
h	Hour(s)
H_2O_2	Hydrogen peroxide
HK	Histidine kinase
Hph	Hygromycin phosphotransferase
Hpt	Histidine-containing phosphotransfer
HRP	Horse radish peroxidase
Hyg^R	Hygromycin resistant
IAA	Iodoacetamide
kb	Kilobase(s)

Kl	*Kluyveromyces lactis*
KO	Knock-out
LB	Luria-Bertani broth
µg	Microgram
µm	Micrometre
µM	Micromolar
µL	Microlitre
M	Molar
MAPK	Mitogen activated protein kinase
min	Minute(s)
mg	Milligram
Mg	*Magnaporthe grisea*
mL	Millilitre
mm	Millimeter
mM	Millimolar
Mo	*Magnaporthe oryzae*
mRNA	Messenger ribonucleic acid
NA	Numerical aperture
NADH	Nicotinamide adenine dinucleotide (reduced form)
NADPH	Nicotinamide adenine dinucleotide phosphate (reduced form)
NBT	Nitroblue tetrazolium
Nc	*Neurospora crassa*
NCBI	National Centre for Biotechnology Information
n-CRD	N-terminal cysteine-rich domain
ng	Nanogram
NO	Nitric oxide
Nox	NADPH oxidase
OM	Osmotic medium
OSR	Oxidative stress response

Pa	*Podospora anserina*
PAMP	Pathogen-associated molecular pattern
PCR	Polymerase chain reaction
PD	Potato dextrose
PEG	Polyethylene glycol
Phox	Phagocyte oxidase
pmol	Picomole
PMSF	Phenylmethylsulfonyl fluoride
Prx	Peroxiredoxin
PTP	Protein tyrosine phosphatase
RG	Regeneration
RNA	Ribonucleic acid
RNase	Ribonuclease
ROS	Reactive oxygen species
rpm	Revolutions per minute
RR	Response regulator
RT	Reverse transcriptase
RT-PCR	Reverse transcriptase-polymerase chain reaction
Sak	Stress-activated kinase
Sc	*Saccharomyces cerevisiae*
SC	Synthetic complete
SD	Synthetic defined
SDS	Sodium dodecyl sulfate
SDS-PAGE	Sodium dodecyl sulfate-polyacrylamide gel electrophoresis
SEM	Scanning electron microscopy
SLS	Sodium lauroyl sarcosine
SOD	Superoxide dismutase
Sp	*Schizosaccharomyces pombe*
Ss	*Sclerotinia sclerotiurum*

TBE	Tris-boric acid-EDTA
tBLASTn	Translated nucleotide database search using a protein query
TEF	Translation elongation factor
TEM	Transmission electron microscopy
Tpx	Thiol peroxidase
Trx	Thioredoxin
U	Unit
Um	*Ustilago maydis*
UTR	Untranslated region
UV	Ultraviolet
V	Volts
v/v	Volume/volume ratio
WGD	Whole genome duplication
WT	Wild-type
w/v	Weight/volume ratio
YE	Yeast extract
YRE	Yap1 response element
°C	Degrees Celsius