

# **Role of Tem1 in Signalling Mitotic Exit in the Human Fungal Pathogen *Candida albicans***

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STEPHEN W. MILNE

## Abstract

The human pathogen *Candida albicans* is polymorphic, and its ability to switch growth forms is thought to play an important role in virulence. The primary research aim of this thesis was to understand the role the mitotic exit network plays in *C. albicans* with particular focus on the Tem1 GTPase protein. This aim was split into three specific goals; to study the role of Tem1 through the construction of a regulatable *tem1* mutant, to understand the regulation of Tem1 through localisation and protein interaction studies, and to construct new molecular tools utilising the *NAT1* positive selection marker in order to achieve two previous goals.

In this thesis we demonstrated that *TEM1* is an essential gene in *C. albicans*, and its essential function is signalled through the Cdc15 protein. Surprisingly, Tem1p depleted cells arrested as hyper-polarised filaments containing one or two nuclei and ultimately lost viability. These filaments formed from budding yeast cells, suggestive of a blockage late in the cell cycle. Ultimately the failure of these filaments to undergo cytokinesis was linked to a defect in septin ring dynamics and the formation of actomyosin ring.

To understand the regulation of Tem1 we localised both the Tem1 and Lte1 proteins and found that Tem1 localised to spindle pole bodies in a cell-cycle dependent fashion by recruited at the onset of S phase. In contrast, the Lte1 homolog localised to the daughter cell cortex prior to release into the cytoplasm at the end of the cell cycle. A yeast 2-hybrid analysis of the MEN components demonstrated the potential of Bfa1/Bub2 and Tem1 to form a complex and the ability of Tem1 to homodimerise which may play a role in its self-activation.

In order to carry out various aspects of this work we constructed a fully functional set of cassettes, including the constitutively active *ENO1* promoter, V5-6xHIS epitope tag and various fluorescent protein genes fused to the *NAT1* positive selection marker.

When considered together, these results indicate that Tem1 is required for timely mitotic exit and cytokinesis in *C. albicans*, similar to *S. cerevisiae*, but the final output of the pathway must have diverged.

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**Abbreviations**

ALS	Agglutinin-like sequence
<i>A. nidulans</i>	<i>Aspergillus nidulans</i>
APC	Anaphase promoting complex
APC <sup>Cdc20</sup>	Anaphase promoting complex-Cdc20 complex
APC <sup>Cdh1</sup>	Anaphase promoting complex-Cdh1 complex
<i>A. thaliana</i>	<i>Arabidopsis thaliana</i>
BFP	Blue fluorescent protein
<i>C. albicans</i>	<i>Candida albicans</i>
<i>C. glabrata</i>	<i>Candida glabrata</i>
cAMP	Cyclic adenosine monophosphate
CAR	Contractile actomyosin ring
CDK	Cyclin-dependant kinase
CFP	Cyan fluorescent protein
CFW	Calcofluor white
ChIP-chip	Chromatin immunoprecipitation
DAPI	4,6-diamidino-2phenylindole-dihydrochloride
DIC	Differential interference contrast microscopy
DNA	Deoxyribonucleic acid
Dox	Doxycycline
DTT	Dithiothreitol
<i>E. coli</i>	<i>Escherichia coli</i>
Em	Emission
EML	Extracellular matrix layer
END	Emi1/NuMA/dynein-dynactin
<i>ENO1p</i>	Enolase promoter
Ex	Excitation
FCS	Foetal calf serum
FEAR	Cdc fourteen early anaphase release network
5-FOA	5-Fluoro-orotic acid
GAP	GTPase activating proteins
GDI	Guanosine nucleotide dissociation inhibitor
GEF	Guanosine exchange factor
GFP	Green fluorescent protein
<i>G. mellonella</i>	<i>Galleria mellonella</i>
GlcNAc	<i>N</i> -acetylglucosamine
GPI	Glycophosphatidylinositol
GDP	Guanosine diphosphate
GTP	Guanosine triphosphate
HA	Haemagglutinin
HeLa	Human cervical epithelial
HyB	Hygromycin B

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## Abbreviations

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LiOAc/ssDNA/PEG	Lithium acetate/single stranded DNA/polyethylene glycol
MBF	<i>MluI</i> cell cycle box binding factor
MEN	Mitotic exit network
Mnt	Mannosyltransferase
MPA	Mycophenolic acid
mSPB	Mother-bound spindle pole body
MUAG	4-methylumbelliferyl- <i>N</i> -acetyl-D-glucosamine
MTL	Mating type locus
<i>NAT1</i>	Nourseothricin acetyltransferase
<i>N. crassa</i>	<i>Neurospora crassa</i>
NLS	Nuclear localisation sequence
Nt	Nucleotide
OMPD	Orotidine-5'-phosphate
ORF	Open reading frame
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
PKA	Protein kinase A
Pmt	Protein-O-mannosyltransferase
pNP-GlcNAc	<i>p</i> -nitrophenyl- $\beta$ - <i>N</i> -acetylglucosamine
PP2A <sup>Cdc55</sup>	Protein phosphatase 2A-Cdc55 complex
pYES2.1	Yeast expression system plasmid
RFP	Red fluorescent protein
RHE	Reconstituted human epithelium
RT-PCR	Reverse transcriptase PCR
SAC	Spindle assembly checkpoint
SAP	Secreted aspartic proteinases
<i>SAT1</i>	Streptoethricin acetyltransferase
SBF	Swi4/6 cell cycle box binding factor
SC	Synthetic complete media
<i>S. cerevisiae</i>	<i>Saccharomyces cerevisiae</i>
SDS	Sodium dodecyl sulphate
SEM	Scanning electron microscopy
<i>S. pombe</i>	<i>Schizosaccharomyces pombe</i>
SPB	Spindle pole bodies
dSPB	Daughter spindle pole body
START	G <sub>1</sub> /S checkpoint
TAP	Tandem affinity purification
TBS	Tris buffered saline
TBS-T	TBS plus Tween-20
TBT-T+BSA	TBS-T plus 5 % BSA
TEM	Transmission electron microscopy
TetO	Tet operator sequence
TetR	Tetracycline repressor protein

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## Abbreviations

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v/v	Volume/volume
w/v	Weight/volume
Y2H	Yeast 2-hybrid
YFP	Yellow fluorescent protein
YPD	Yeast extract peptone dextrose
YPGal	Yeast extract peptone galactose