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Analysis of *Epichloë festucae* membrane lipid  
composition and its role in Nox complex  
assembly

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## Abstract

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*Epichloë festucae* is a filamentous fungus that forms a highly regulated mutualistic symbiosis with perennial ryegrass. The spatially and temporally controlled production of reactive oxygen species (ROS) by the fungal NADPH oxidase (Nox) complex regulates this interaction by restricting fungal growth *in planta*. Whilst much is known about the importance of ROS in plant-fungal interactions, comparatively little is known about how its production is regulated. In plant and mammalian systems, production of ROS by the Nox complex is regulated via lipid signalling. Cytosolic Nox components containing lipid-binding domains are targeted to specific phosphoinositide enriched at certain locations within in the plasma membrane. Once assembled, specific lipids then directly activate the Nox complex. It is hypothesised that similar regulation also occurs in fungi. This study investigated whether lipid signalling could play a role in regulating the fungal Nox complex. The lipid-binding PH domain of Nox protein Cdc24 was found to be necessary for membrane localisation, supporting a role for lipid signalling in fungal Nox complex regulation. To identify potential lipid targets for the cytosolic Nox proteins, a comprehensive analysis of *E. festucae* membrane lipid composition was carried out using a suite of biosensors. These biosensors consisted of mammalian lipid binding domains of known specificity fused to a fluorophore, enabling live cell imaging of phosphoinositide localisation both in culture and *in planta* via fluorescence microscopy. Phosphatidylinositol 4,5-bisphosphate was detected in the plasma membrane and septa in culture and *in planta*. A striking asymmetric gradient was observed at the hyphal tip, with enriched fluorescence in the sub-apical region. Similar to yeast, phosphatidylinositol 4-phosphate was localised to golgi vesicles in culture. In contrast, phosphatidylinositol 3-phosphate was found in vacuolar and endosomal membranes. Biosensors for phosphatidylinositol 3,4-bisphosphate and phosphatidylinositol 3,4,5-triphosphate, localised to the cytoplasm in culture and *in planta*, suggesting that these phospholipids were absent under the growth conditions examined. These results confirm a role for lipid signalling in fungal Nox complex assembly and provide insight into membrane lipid composition, identifying candidate phosphoinositide targets for assembly.



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## List of abbreviations

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Amp	ampicillin
Amp <sup>R</sup>	ampicillin resistant
bp	base pair
BLAST	basic local alignment search tool
BLASTp	search of protein databases with a protein query
cDNA	complementary DNA
Cp	crossing point
CR	contractile ring
d	day(s)
DIC	differential interference contrast
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
dNTP	deoxynucleotide triphosphate
EDTA	ethylenediaminetetraacetic acid
eGFP	enhanced green fluorescent protein
EM	electron microscopy
g	gram
gDNA	genomic DNA
Gen	geneticin
Gen <sup>R</sup>	geneticin resistant
H	hour
Hyg	hygromycin
Hyg <sup>R</sup>	hygromycin resistant
HPLC	high performance liquid chromatography
Kb	kilobase
L	litre
LBD	lipid-binding domain
LB	Luria-Bertani
M	Molar
MAP	mitogen-activated protein
µg	microgram

μL	microlitre
μm	micrometre
μM	micromolar
mg	milligram
mL	millilitre
mm	millimetre
mM	millimolar
min	minute
mRNA	messenger RNA
MVB	multi-vesicular body
N/A	not applicable
NADPH	nicotinamide adenine dinucleotide phosphate
Ng	nannogram
nM	nannomolar
Nox	NADPH oxidase
OE	overexpression
PCR	polymerase chain reaction
PD	potato dextrose
PEG	polyethylene glycol
PIK	phosphatidylinositol kinase
PI[3]P	phosphatidylinositol 3-phosphate
PI[3,4]P <sub>2</sub>	phosphatidylinositol 3,4-bisphosphate
PI[3,4,5]P <sub>3</sub>	phosphatidylinositol 3,4,5-triphosphate
PI[4]P	phosphatidylinositol 4-phosphate
PI[4,5]P <sub>2</sub>	phosphatidylinositol 4,5-bisphosphate
PM	plasma membrane
PPI	phosphoinositide
qRT-PCR	quantitative reverse transcription PCR
RG	regeneration
RNA	ribonucleic acid
ROS	reactive oxygen species
rpm	revolutions per minute
RT-PCR	reverse transcriptase PCR

s	second
SDS	sodium dodecyl sulphate
SNX	sorting nexin
spp.	species
TBE	Tris/Borate/EDTA
tBLASTn	search of translated nucleotide databases with a protein query
TGN	<i>trans</i> -Golgi network
UV	ultraviolet
V	volt
v / v	volume to volume
vol	volume
w / v	weight to volume
WT	wild-type

