A conserved signalling network regulates *Epichloë festucae* cell-cell fusion and the mutualistic symbiotic interaction between *E. festucae* and *Lolium perenne.*

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Abstract

Epichloë festucae is a filamentous fungus that forms a mutually beneficial symbiotic association with *Lolium perenne*. The NADPH oxidase complex components *noxA, noxR* and *racA*, the transcription factor *proA*, and the cell wall integrity (CWI) MAP kinases, *mkkA* and *mpkA*, are required for mutualistic *E. festucae-L. perenne* associations and cell-cell fusion. Homologues of these genes in *Neurospora crassa, Sordaria macrospora* and *Podospora anserina* are required for cell-cell fusion and sexual fruiting body maturation, thereby establishing a link between self signalling and hyphal network formation in the *E. festucae-L. perenne* symbiosis. In *Podospora anserina,* IDC2 and IDC3 are required for cell-cell fusion, crippled growth and fruiting body formation. In *S. macrospora* and *N. crassa,* components of the STRIPAK complex regulate cell-cell fusion and fruiting body formation. The aim of this project was to test if *E. festucae* homologues of IDC2 and IDC3, and the STRIPAK complex protein MOB3, named SymB, SymC and MobC, respectively, are also required for cell-cell fusion and plant symbiosis. Gel shift assays showed the promoters of *symB* and *symC* are targets for the transcription factor ProA. In culture, the frequency of cell-cell fusion of Δ*mobC* was reduced, but in Δ*symB* and Δ*symC* mutants, totally abolished. All three mutants hyperconidiated and formed intra-hyphal hyphae. Plants infected with these mutants were severely stunted and hyphae exhibited proliferative growth and increased colonisation of the intercellular spaces and vascular bundles. Expressoria formation, structures allowing colonisation of the leaf surface, was reduced in Δ*mobC,* and abolished in Δ*symB* and Δ*symC* mutants. Microscopy analyses showed SymB-GFP and SymC-mRFP1 co-localise to the plasma membrane and septa. SymC also localised to highly dynamic punctate structures. Although Δ*symB* and Δ*symC* phenotypes are identical to Δ*mpkA,* and the *E. festucae* pheromone response pathway scaffold Δ*idcA* mutants, MpkA and MpkB phosphorylation and cellular localisation was unchanged compared to wild-type. Using yeast-two-hybrid assays, an interaction between SymC and the STRIPAK complex associated protein GPI1 was demonstrated. Collectively these results show that MobC, SymB and SymC are required for *E. festucae* cell-cell fusion and host symbiosis. It is proposed that SymB and SymC interact to form a sensor complex at the cell wall which regulates cell-cell fusion in culture and hyphal network development *in planta*.

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Abbreviations

aa	Amino acid
Amp	Ampicillin
Amp^R	Ampicillin resistant
Ao	*Aspergillus oryzae*
asc	Ascogonia mutant
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)
CAT(s)	Conidial anastomosis germ tube(s)
cDNA	Complementary DNA
CFW	Calcofluor white
CG	Crippled growth
CIAP	Calf intestinal phosphatase
CLSM	Confocal Laser Scanning Microscopy
Cp	*Claviceps purpurea*
CW	Cell wall
CWI	Cell wall integrity
Cys	Cysteine
DIC	Differential interference contrast
DIG	Digoxigenin
DNA	Deoxyribonucleic acid
dNTP	Deoxynucleotide triphosphate
EDTA	Ethylene diamine tetra-acetic acid

eGFP	Enhanced green fluorescent protein
EMSA	Electrophoretic mobility shift assay
FB	Fruiting body
Fg	*Fusarium graminearum*
Fo	*Fusarium oxysporum*
g	Gram
gDNA	Genomic DNA
GEF	Guanine nucleotide exchange factor
Gen	Geneticin
Gen^R	Geneticin resistant
GFP	Green fluorescent protein
GPI	Glycosylphosphatidylinositol
h	Hour(s)
ham	Hyphal anastomosis mutant
H_2O_2	Hydrogen peroxide
Hph	Hygromycin
Hyg^R	Hygromycin resistant
IDC	Impaired development of crippled growth
kb	Kilobase(s)
KO	Knock-out
L	Litre
LB	Luria-Bertani broth
M	Molar
MAPK(K/K)	Mitogen activated protein kinase (kinase/kinase)
mg	Milligram
Mg	*Magnaporthe grisea*
µg	Microgram
min	Minute(s)
µL	Microlitre

mL	Millilitre
µm	Micrometre
µM	Micromolar
mm	Millimeter
mM	Millimolar
Mo	*Magnaporthe oryzae*
MOB	Monopolar spindle-one-binder
mRFP1	Monomeric red fluorescent protein
mRNA	Messenger ribonucleic acid
NADH	Nicotinamide adenine dinucleotide (reduced form)
NADPH	Nicotinamide adenine dinucleotide phosphate (reduced form)
Nc	*Neurospora crassa*
NCBI	National Centre for Biotechnology Information
ng	Nanogram
Nox	NADPH oxidase
Pa	*Podospora anserina*
PB1	Protein binding domain 1
PC	Plant cell
PCR	Polymerase chain reaction
PD	Potato dextrose
PEG	Polyethylene glycol
per	Perithicia mutant
pile	Perithicia placement mutant
pg	Picogram
pmol	Picomole
PMSF	Phenylmethylsulfonyl fluoride
PP2A	Protein phosphatase 2A
PR	Pheromone response
pro	Protoperithicia mutant

RG	Regeneration
RNA	Ribonucleic acid
RNase	Ribonuclease
RNA-seq	Ribonuclease sequencing
ROS	Reactive oxygen species
rpm	Revolutions per minute
RT	Reverse transcriptase
RT-PCR	Reverse transcriptase-polymerase chain reaction
SAK	Stress-activated kinase
SAM	Shoot apical meristem
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
STRIPAK	Striatin interacting phosphatase and kinase
sym	Symbiosis mutant
TBE	Tris-boric acid-EDTA
tBLASTn	Translated nucleotide database search using a protein query
T-DNA	Transfer-deoxyribonucleic acid
TEM	Transmission electron microscopy
TMD	Transmembrane domain
Um	*Ustilago maydis*
UV	Ultraviolet
V	Volts
v/v	Volume/volume ratio
WT	Wild-type

w/v	Weight/volume ratio
YE	Yeast extract
°C	Degrees Celsius