Transcriptional regulation during appressorium formation and function in *Glomerella cingulata*

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Abstract

Glomerella cingulata, anamorph *Colletotrichum gloeosporioides*, causes bitter rot in apples and fruit rot in other subtropical fruits. In response to environmental cues such as contact with the host, *Glomerella cingulata* forms a special structure called an appressorium, which accumulates glycerol and thereby generates a sufficiently high turgor pressure to push an infection peg into the host tissue. It is known that the cAMP and MAPK signalling transduction pathways control appressorium formation and function in *Colletotrichum* species and other appressorium-forming fungi. This process is accompanied by a global change in gene expression. Little is known of transcriptional regulation during this process. The aim of this project was to study the transcriptional regulation of appressorium formation and function in *G. cingulata.*

The *G. cingulata SAP* gene had previously been shown to be expressed as a longer transcript during the early stage of appressorium differentiation. It was considered possible that the transcription factors that regulated expression of the longer transcript may be also involved in the regulation of appressorium differentiation. Identification of the transcription factor involved may help to understand the mechanisms that regulate appressorium differentiation. The plan was to use the yeast one-hybrid system to isolate the transcription factor. This required identification of the promoter regions responsible for expression of the longer *SAP* transcript. Therefore, the *G. cingulata SAP* promoter was characterized by mapping the transcription start point. Three transcription start points were determined by RLM-RACE. To further characterise the promoters, *SAP-GFP* reporter plasmids were constructed and transformed into *G. cingulata.* Even though a reasonable level of *GFP* expression was observed in RT-PCR experiments, however, no differences in fluorescence intensity were seen between the wild type and GFP reporter transformants. Therefore, no further attempts to study the *sap* promoter were made.

The candidate gene approach was chosen as an alternative way to study the transcriptional regulation of appressorium formation and function in *G. cingulata.* The

G. cingulata StuA gene was cloned using degenerate PCR, single specific primer PCR, subgenomic library screening and plasmid rescue from a disruption mutant. Targeted gene deletion of the *G. cingulata StuA* gene was successful. Deletion mutants display many phenotypic changes. Complementation mutants were constructed to confirm the function of this gene. A full length copy of this gene together with a second selection marker was reintroduced into the deletion mutant and the wild type phenotype was restored.

Deletion mutants form appressoria at the normal rate and with unaltered morphology. In comparison with the wild type, these appressoria did not generate high turgor pressure as shown by a cytorrhysis assay. This resulted in a defect in appressorium penetration of onion epidermal cells. Nor were these mutants able to invade unwounded apples. Therefore, the *G. cingulata StuA* gene is required for appressorium function. In addition, deletion mutants displayed stunted aerial hyphae, "wettable" mycelium, reduced conidia production, and a defect in conidiophore and perithecium formation. These results suggested that the *G. cingulata StuA* gene has multiple roles in fungal development.

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Abbreviations

bp	base pair
BSA	Bovine serum albumin
cAMP	cyclic AMP
cDNA	complementary deoxyribonucleic acid
CWDE	cell wall degradation enzyme
dATP	deoxyadenosine triphosphate
dCTP	deoxycytosine triphosphate
dNTP	deoxynucleotide triphosphate
DHN	dihydroxynaphthalene
DTT	1,4-dithiothreitol
DIG	Digoxigenin
EDTA	Ethylenediamine tetraacetic acid
ECM	extracellular matrix
GFP	green fluorescence protein
kb	kilobase pair
MAPK	mitogen activated protein kinase
ORF	open reading frame
PCR	polymerase chain reaction
PEG	Polyethylene glycol
PKA	protein kinase A
5' UTR	5' un-translated region
PCR	Polymerase chain reaction
RT-PCR	Reverse transcription PCR
RACE	Rapid amplification of cDNA ends
REMI	restriction enzyme mediated integration
Sec	Second(s)
SDS	Sodium dodecyl sulfate
SSC	sodium chloride/sodium citrate (buffer)