Multiple regulators contribute to the transcriptional control of the Candidalysin-encoding gene *ECE1*

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Introduction

Candidalysin is a toxic peptide which is secreted by *C. albicans* hyphae during the infection of human host cells. The peptide is derived from a precursor protein which is encoded by the *ECE1* gene. *ECE1* is normally the most abundant transcript in hyphae, but it is not expressed in yeast cells. The aim of this study was to identify regulators of *ECE1* transcription.

Visualization of *ECE1* transcription

To establish an *ECE1*-GFP reporter system (p*ECE1*-GFP), one *ECE1* allele was replaced by *GFP*. The strain was then grown for 6h at 37°C in either SDG or SDG with 10% human serum (HS). Only hyphae showed a GFP signal. This observation correlates with the qRT PCR results.

Transcriptional regulators Ahr1 and Tup1 are required for high level expression of *ECE1*

p*ECE1*-GFP in activator mutants

The indicated strains were grown in SDG+HS for 6h at 37°C prior to microscopy. The repressor mutants were also incubated in SDG (6h, 37°C). Scale bar: 20μm.

p*ECE1*-GFP in repressor mutants

Low *ECE1* expression in *ahr1Δ* and *tup1Δ* mutants

RNA was isolated after 6h growth in SDG+HS at 37°C. Gene expression was normalized against *ACT1* and a control RNA (5h YPD, 37°C). Asterisks mark significant differences ($p \leq 0.05$, two-tailed, unpaired student's *t*-test).

Stimulus-independent activation of *ECE1* by a hyperactive *AHR1* allele

A hyperactive *AHR1* allele (*AHR1*-GAD) triggers expression of *ECE1* without additional stimuli. This up-regulation can also be observed in various regulatory mutants like *cph1Δ/efg1Δ*. Interestingly, the presence of Tup1 was required for this positive effect.

The indicated strains were grown in SDG+HS for 6h at 37°C prior to microscopy or RNA isolation. Scale bar: 20μm. Gene Expression was normalized as mentioned above.

Mcm1 is involved in the regulation of *ECE1*

Mcm1 is a known interacting partner of Ahr1. An overexpression of this regulatory gene (MCM1^{OE}) leads to increased *ECE1* mRNA amounts. However, this effect was significantly lower in *ahr1Δ*.

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Summary

The transcriptional control of *ECE1* is complex and includes multiple regulators. Our data showed that Ahr1 and Tup1 contribute to the activation of the gene, indicating that Tup1 is not solely a repressor. In addition, we were able to show that Mcm1 might be involved in the activation of *ECE1*. Further experiments will examine if these three regulators act together during the transcriptional control of *ECE1*.

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