30-4 - Das H3K4 Methyltransferasegen *KMT2* ist ein neuer Virulenzfaktor des Mais Anthraknose Pathogens *Colletotrichum graminicola*

The H3K4 methyltransferase gene KMT2 is a novel virulence factor of the maize anthracnose pathogen Colletotrichum graminicola

Marvin Florian Sonnek[1], Alan de Oliveira Silva[1], Jan Grau[2], Holger B. Deising[1]

[1]Martin-Luther-University Halle-Wittenberg, Faculty of Natural Sciences III, Phytopathology and Plant Protection.
[2]Martin-Luther-University Halle-Wittenberg, Faculty of Natural Sciences III, Institute of Computer Science

The histone-methyltransferase gene *KMT2* is responsible for mono-, di- and trimethylation of lysine K4 of histone H3 (H3K4), as first described as *SET1* in *Saccharomyces cerevisiae*. The gene is evolutionarily highly conserved and encodes the enzymatically active subunit *Set1* of a protein complex called Compass. This complex transfers methyl-groups to H3K4 and contributes to the establishment of transcriptionally active euchromatin, thus playing an important role in epigenetic transcriptional regulation.

The ascomycete *Colletotrichum graminicola* is a hemibiotrophic plant pathogen that penetrates and colonizes its host plant through an appressorium. The pathogen then forms a primary biotrophic hypha in the first host cell, which subsequently differentiates into secondary necrotrophic hyphae, necrotizing the surrounding host tissue and causing anthracnose disease symptoms. As differentiation of infection structures requires significant transcriptional re-programming, epigenetic regulatory factors may play a key-role in pathogenic development.

We used homologous recombination to generate Δ*kmt2* mutants of the maize anthracnose fungus. Employing antibodies targeting H3K4me1, H3K4me2 and H3K4me3, we confirmed the absence of these three marks in Δ*kmt2* mutants by Western Blot and immuno-cytological analyzes, the latter also showing uniform labeling of euchromatin. In comparison to the wild-type strain (WT), the mutants produced significantly smaller conidia, and severe defect in hyphal growth and reduced asexual conidiation were observed. Importantly, infection assays on *Zea mays* revealed defects in appressorial penetration on host leaves, and few appressoria that were able to penetrate the epidermal host cell were developmentally arrested and unable to colonize the plant.

Our data show that H3K4 methylation plays a significant role in pathogenic development of *C. graminicola* and identified *KMT2* as a novel virulence factor.

Finanzierung: ESF

30-5 - Der Einfluss von XPP1 auf die Xylan Degradation durch den Maisanthracnose verursachenden Pilz *Colletotrichum graminicola*

The role of XPP1 in xylan degradation by the maize anthracnose pathogen Colletotrichum graminicola

Renata Amorim, Alan de Oliveira Silva, Anja Raschke, Holger B. Deising

Martin-Luther-University Halle-Wittenberg, Faculty of Natural Sciences III, Phytopathology and Plant Protection

Colletotrichum graminicola is a hemibiotrophic fungal pathogen that causes maize anthracnose disease. Initial penetration of the plant cuticle and cell walls is mediated by an infection cell called appressorium, from which biotrophic hyphae evolve. The biotrophic development ends when necrotrophic hyphae are formed. These hyphae rapidly colonize the host tissue, cause cell death and occurrence of anthracnose disease symptoms. The transition to the necrotrophic phase has been associated with increased production of cell wall-degrading enzymes, such as xylanases, which are possibly utilized for both for host

colonization and for generation of simple sugars used for nutritional purposes. Thus xylanases are thought to play an important role in fungal pathogenesis. The gene XPP1 – Xylanase promoter binding protein 1 – was first described as a xylanase regulator in the filamentous ascomycete Trichoderma reesei, acting not only as a regulator of xylanase gene expression, but also as a repressor of secondary metabolism. We identified a XPP1 homologue in the maize anthracnose pathogen C. graminicola (CgXPP1) and obtained deletion mutants. By comparing these mutants with the wild type strain, we aim to investigate the role of XPP1 in the maize pathogenesis. Interestingly, first results show that in XPP1-deficient mutants a shift in transcript abundance of the xylanase transcription factor XLR1 occurs. We are currently studying the role of XPP1 and XLR1 in growth of vegetative hyphae on simple and polymeric carbohydrates, as well as in virulence.

Finanzierung: ESF

30-6 - Die Bedeutung von CRE1 und SNF1 für die Synthese Zellwand abbauender Enzyme und Virulenz des Mais Anthraknose Pathogens *Colletotrichum graminicola*

Roles of CRE1 and SNF1 in production of cell wall-degrading enzymes (CWDE) and full virulence of the maize anthracnose pathogen Colletotrichum graminicola

Alan de Oliveira Silva[1], Chirlei Glienke[2], Holger B. Deising[1]

[1]Martin-Luther-University Halle-Wittenberg, Faculty of Natural Sciences III, Phytopathology and Plant Protection
[2]Federal University of Paraná, Center of Biological and Health Sciences, Department of Genetics

Colletotrichum graminicola causes leaf anthracnose and stalk rot in maize. The pathogen differentiates an infection cell called an appressorium in order to invade the epidermal maize cell in a turgor pressure-driven fashion, possibly supported by secreted cell wall-degrading enzymes (CWDEs), which have previously been associated with virulence in different filamentous fungi. However, due to their enormous number and functional redundancy, targeted deletion of individual CWDE genes rarely caused virulence defects, and their role in fungal infection remains poorly understood. As an alternative to deletions of individual CWDE genes, targeted mutagenesis of genes controlling the expression of many genes encoding CWDEs may improve understanding the function of these enzymes during pathogenesis. The *CRE1* gene codes for a transcriptional repressor recognizing a conserved motif in the promoter region of many CWDEs genes. With opposite effect, the *SNF1* gene encodes a serine-threonine protein kinase required for phosphorylation of Cre1 and de-repression of genes subject to catabolite repression.

In this study we identified and functionally characterized *CRE1* and *SNF1* of *C. graminicola*. In comparison to the wild-type strain (WT), deletion mutants for both genes showed impaired vegetative growth and asexual sporulation, as well as penetration rates in maize leaves, as the mutants were significantly affected in their ability to cause anthracnose symptoms. In Δ*snf1* mutants, reduced growth rates only occurred on polymeric carbon sources, but not on simple sugars such as glucose and sucrose. The homologue of the xylanase transcription factor *XLNR* of *Aspergillus oryzae*, harboring a Cre1 binding site, was repressed in Δ*snf1* mutants and overexpressed in Δ*cre1* mutants even even in the presence of simple sugars. The fact that both Δ*cre1* and Δ*snf1* mutants exhibit strongly reduced virulence highlights the role of catabolite repression and de-repression in the hemibiotroph *C. graminicola*.

Finanzierung: ESF