The role of the F-Box protein Frp1 in pathogenicity of fusarium oxysporum
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Previously, FRP1 has been identified in a mutant screen as a pathogenicity gene of the plant pathogenic fungus *Fusarium oxysporum* f.sp. *lycopersici*. Deletion of the gene confirmed the requirement of FRP1 for pathogenicity. FRP1 encodes an F-box protein and in this thesis we set out to elucidate its molecular function further. Literature on fungal F-box proteins in general is reviewed in Chapter II and in Chapter III the F-box protein arsenal of four *Fusarium* species is analyzed by mining their genome sequences. Furthermore, the function of the Frp1 F-box domain is studied (Chapter IV), the phenotype of the Δfrp1 mutant strain examined (Chapter V) and the involvement of CreA in Frp1 function investigated (Chapter VI).

Many F-box proteins play a key role in the degradation of other proteins. They recruit such proteins to a ubiquitination machine called the SCF complex where they become ubiquitinated, which commonly leads to recognition and degradation by the proteasome. Binding to Skp1, a component of the SCF complex, is a strong indication that Frp1 recruits targets to this complex. Identification of such target proteins of Frp1 could give clues about its molecular function(s) and, therefore, several screens were designed to identify a Frp1-target. Unfortunately, no target protein was found. This raised the question whether Frp1 actually recruits target proteins. To investigate this, mutants of Frp1 were created with mutations in the F-box domain that abolished the binding of Frp1 to Skp1. If Frp1 would function through binding to Skp1, then it would be expected that these mutations would affect the function of Frp1 and, hence, pathogenicity. However, this appeared not to be the case as the Skp1-nonbinding versions of Frp1 could restore pathogenicity of the Δfrp1 mutant strain. This suggests that the main function of Frp1 may not be recruitment of other proteins to an SCF complex (Chapter IV).

To find out why the Δfrp1 mutant is unable to cause disease, its phenotype was investigated in more detail. We found that the mutant is impaired in root colonization as well as in root invasion. These defects are accompanied by impaired growth on a broad array of alternative (non-sugar) carbon sources such as organic acids, amino acids and polysaccharides. We also found that the production of cell wall degrading enzymes (CWDE) is seriously affected in the Δfrp1 mutant, which is likely related to the reduced growth on polysaccharides. The colonization defects on tomato roots could be restored by addition of sugars like glucose or sucrose. However, addition of sugars could not restore
the root invasion defects as shown by the lack of expression of the GFP-reporter gene behind the SIX1-promoter (the SIX1 gene is specifically expressed inside roots and is therefore a marker for root invasion). To find out whether reduced growth on C2-carbon sources like ethanol was (partly) responsible for the non-pathogenic phenotype of the Δfrp1 mutant, we created an ICL1 deletion mutant. Icl1 is a key enzyme of the glyoxylate cycle, which is required for the assimilation of C2-carbon sources. We found that this mutant -like the Δfrp1 mutant- is impaired in growth on organic acids, ethanol and fatty acids. However, growth on polysaccharides was normal and this mutant was still able to colonize roots and fully cause disease on tomato plants. Therefore we concluded that the lack of CWDE gene expression is sufficient to explain the loss of pathogenicity of the Δfrp1 gene, although other factors, such as utilization of amino acid as a carbon source, could not be ruled out (Chapter V).

The genes required for assimilation of the carbon sources on which the Δfrp1 mutant shows impaired growth are generally under carbon catabolite repression. In fungi, this process is regulated by the transcriptional repressor CreA. We considered the possibility that Frp1 is required for the derepression of these genes by directly or indirectly regulating CreA. The relation between CreA and Frp1 was therefore investigated and we found that the two proteins together control the expression of CreA-repressed genes (Chapter VII). Whereas CreA is mainly (but not exclusively) responsible for repression, Frp1 is required for activation of such genes. Although knock-out of CREA failed, we were able to make mutants in which GST-tagged CREA, had replaced the native CREA gene. Apparently, the GST-tag on CreA affects CreA function since replacement of native CREA by GST::CREA in the wild type as well as in the Δfrp1 mutant background caused a general growth reduction and the inability to repress gene expression during growth on glucose. Remarkably, GST::CREA restored the ability to express CWDE genes in the Δfrp1 mutations, as well as growth on alternative carbon sources and infection of tomato. How Frp1 influences CreA and activates gene expression remains unknown, but it probably does not so via SCF-mediated protein ubiquitination, as explained in Chapter IV. We also could not demonstrate a direct interaction between Frp1 and CreA. Further, accumulation of GFP-tagged CreA in the nucleus occurred both on glucose and on ethanol, a derepressing carbon source, and was not affected by deletion of FRP1.

In conclusion, analysis of the function of the F-box protein Frp1 in the plant pathogenic fungus Fusarium oxysporum revealed that it functions independently from an SCF complex, it is involved in carbon catabolite derepression and, together with CreA, affects expression of CWDE genes during infection.