Feedback Regulation Of The Pmk1 Mitogen-Activated Protein Kinase Pathway During Infection-Related Development In The Rice Blast Fungus

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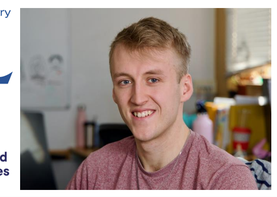


Background
- Yearly 30% of global rice crop yields are lost due to blast disease caused by *Magnaporthe oryzae*
- Pathogenicity Mitogen activated protein Kinase 1 (Pmk1) is essential for *M.oryzae* infection-related development
- Pmk1-dependent phosphosites have been identified in the upstream activating kinases Mst7 and Mst11

Objective
- Determining detailed regulation of the Pmk1 cascade at molecular level

Summary
- Phosphorylation of Ser358 in Mst7 is shown to negatively regulate the Pmk1 cascade

Significance
- These results demonstrate a novel mode of regulation for the Pmk1 pathway during infection-related development

M. oryzae infection cycle

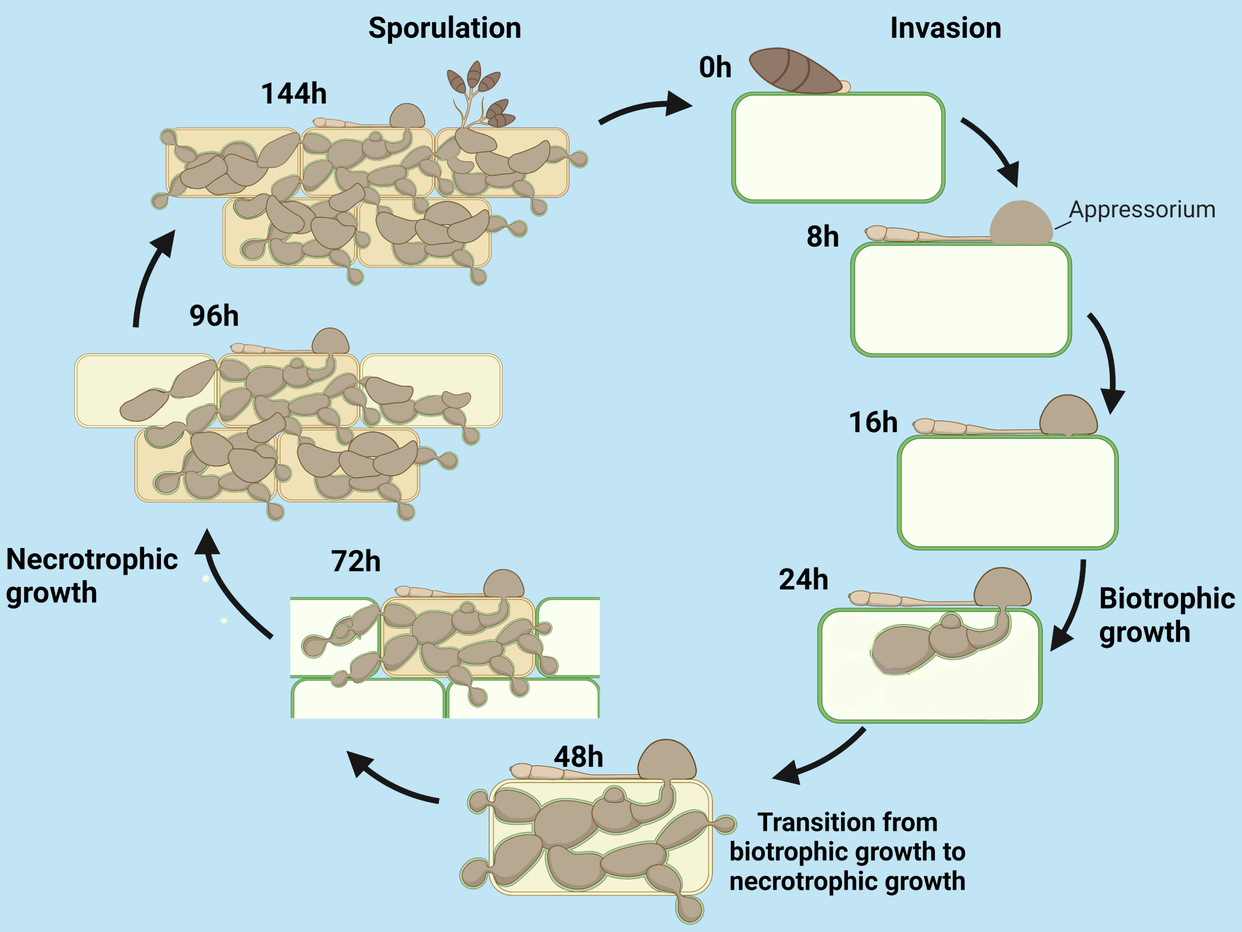


Figure 1. A schematic representation of different developmental stages of the *M. oryzae* life cycle during infection of a rice plant. A specialised cell, called an appressorium, is required for penetration of the leaf surface, essential for successful infection of the host and completing the life cycle.

Pmk1 is a key regulator of appressorium formation

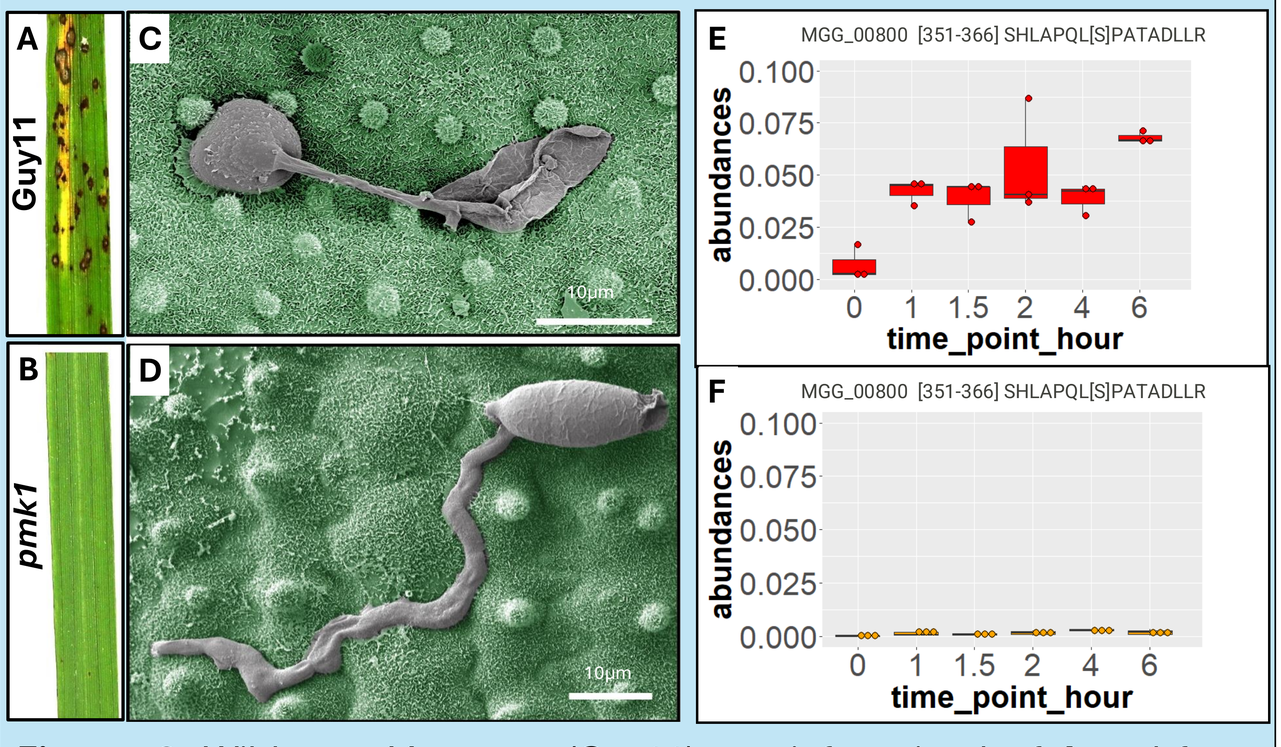


Figure 2. Wild type *M. oryzae* (Guy11) can infect rice leaf **A** and form appressoria **C.** Pmk1 null mutant (*pmk1*) fails to infect rice leaf **B** or form appressoria **D**. A phosphosite in Mst7 is detected in wild type *M. oryzae* **E** but not in *pmk1* mutant **F** suggesting this site is a target of Pmk1 phosphorylation. Cruz-Mireles et al., 2024. Phosphorylated residue shown in square brackets.

The phosphomimic Mst7^{S358D} allele abrogates appressorium formation

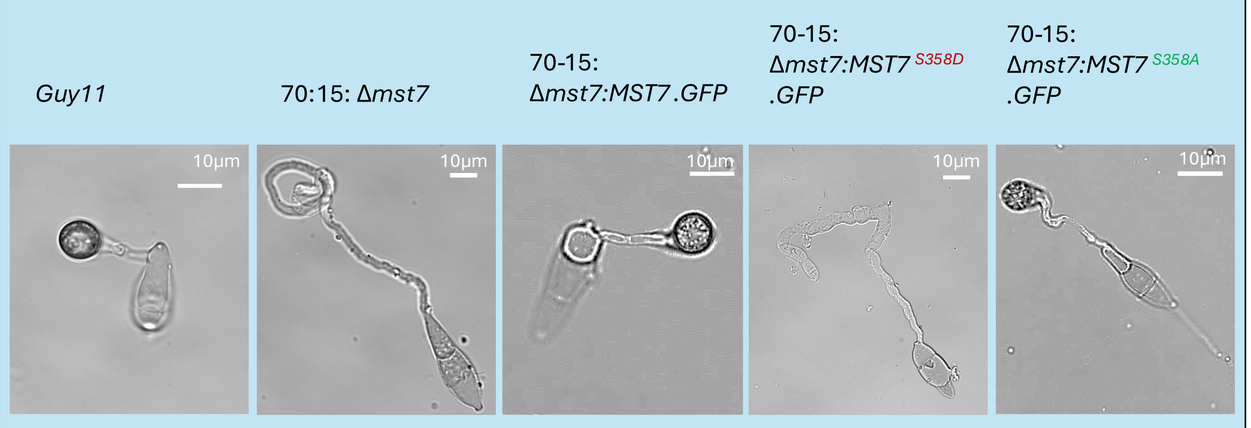


Figure 3. Substitution of a serine residue 358 for a negatively charged aspartate residue mimics phosphorylation of the serine residue. Introducing this substitution to a Pmk1 dependent phosphosite in Mst7 reduced appressorium formation suggesting it is a negative regulator of the Pmk1 cascade. Western blot analysis shows expression of constructs

The Phosphomimetic Mst7^{S358D} shows lower frequency of appressorium formation than wild type

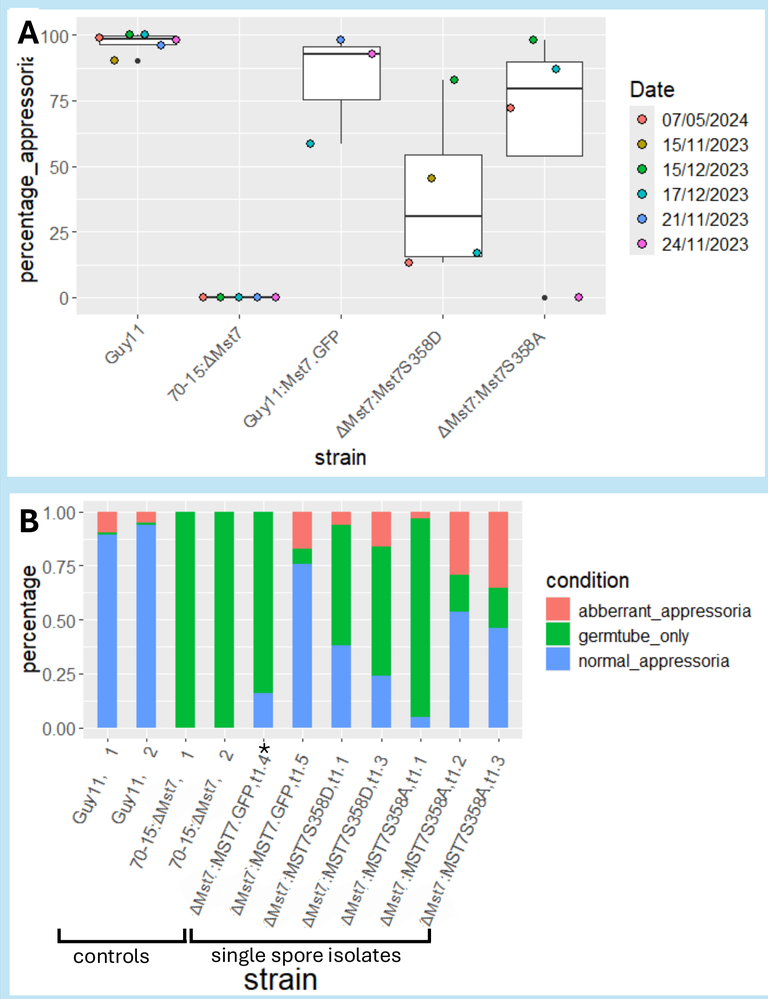
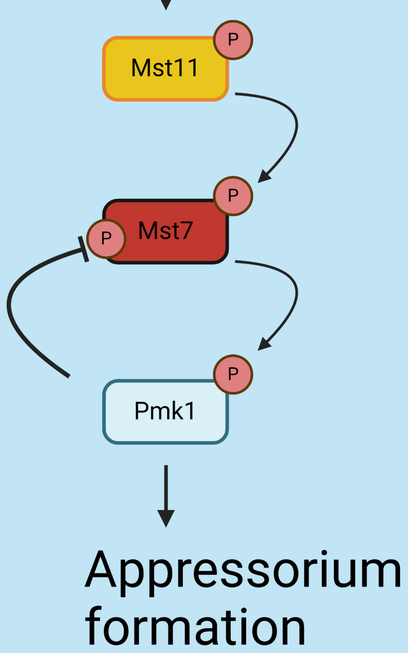


Figure 4. A) Quantification of appressoria formation showed that frequency in the Δ*mst7* complemented with *MST7p:MST7*^{S358D}*:GFP* is lower than wild type. B) Quantification of the frequency of normal appressoria, aberrant appressoria, or germtube only for two to three single spore isolates (t) of complimented Δ*mst7* strains showed a higher frequency of germtube only than wild type for Δ*mst7* complemented with *MST7p:MST7*^{S358D}*:GFP.* Protein expression was assessed by fluorescence microscopy and was lower for one isolate (*). Quantification of controls was performed twice. These data further support the hypothesis that phosphorylation of Serine 358 in Mst7 negatively regulates the Pmk1 cascade.

Conclusion



Future questions

What is the role of other non-canonical Pmk1 dependent phosphosite in Mst7?

Does phosphorylation of Mst7 S358 affect pathogenicity and Pmk1 activity?

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