Characterization of E3 Ubiquitin Ligase 1 as a novel regulator of PTI-associated resistance in *Arabidopsis thaliana*

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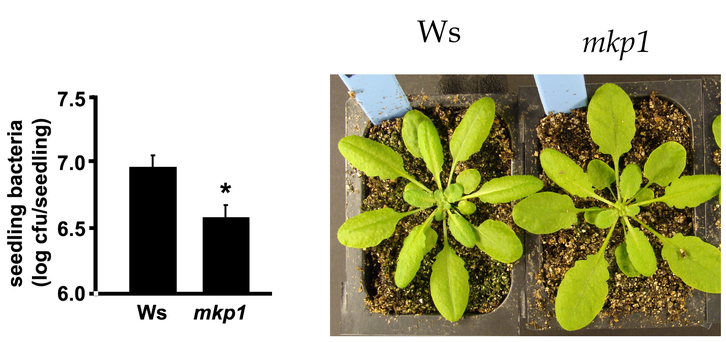
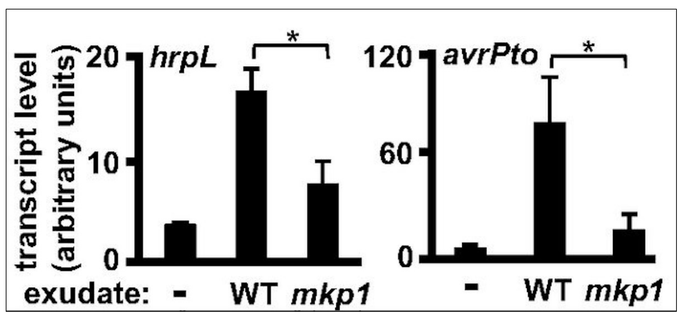
Introduction

E3 Ubiqutin Ligase 1 (E3L1), a plasma membrane-localized E3 Ligase in *Arabidopsis thaliana*, has previously been shown to be rapidly phosphorylated during pattern-triggered immunity (PTI)-associated defense responses. In this work, our characterization of T-DNA insertional mutants (*e3l1-1*, *e3l1-2*) implicate E3L1 as a positive regulator of PTI. The mutant plants are compromised in their ability to manifest PTI-induced resistance as indicated by the failure of pretreatment of *e3l1* mutants with flg22 to manifest reduced bacterial growth of virulent *Pseudomonas syringae* pv tomato DC3000 (Pst DC3000) as observed in wild type plants. However, several other typical defense related phenotypes, including defense transcript accumulation, ROS production, and ligand-induced endocytosis of FLS2, were unaltered, indicating that E3L1 functions downstream or independently of these responses. In a previous study, we demonstrated that loss of MAP Kinase Phosphatase 1 (MKP1) results in increased resistance against Pst DC3000. This resistance was shown to be caused by decreased extracellular accumulation of T3SS-inducing metabolites, indicating alterations in metabolite transporter activity or accumulation in *mkp1* plants. Due to a possible function of E3L1 targeting proteins for degradation or endocytosis at the plasma membrane, we hypothesized a genetic link between E3L1 and MKP1. Infection of a double mutant, *e3l1 mkp1*, shows a suppression of *mkp1*-related resistance, genetically placing E3L1 in the MKP1-dependent pathway. We propose that E3L1 may be responsible for regulating the abundance or activity of the transporter(s) involved in secreting the bioactive metabolites, thus playing a key role during PTI.

Background



mkp1 plants are resistant to *Pseudomonas syringae* pv tomato without a fitness penalty

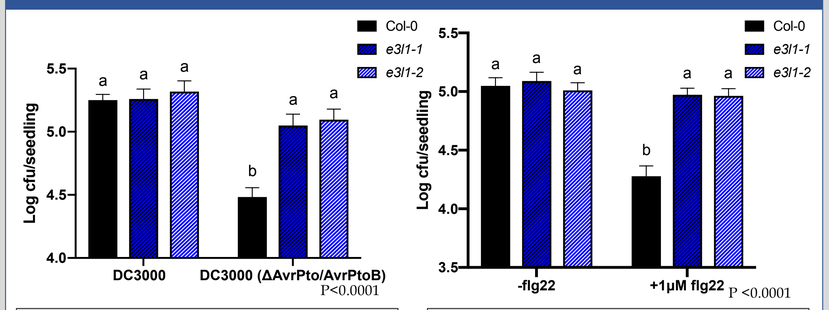


mkp1 plants have a significant decrease in extracellular bioactive metabolites. Bacteria treated with *mkp1* plant exudate do NOT induce their T3SS effectively

Anderson et al (2011) Plant J 67: 258; Anderson et al (2014) PNAS 111:6846

What causes the decrease in extracellular bioactive metabolites?

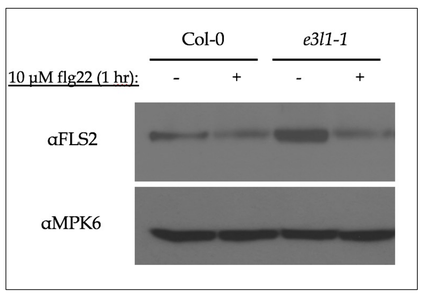
A novel protein, E3L1, is necessary for PTI-induced resistance



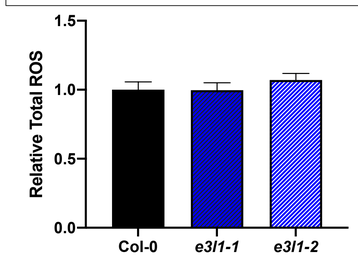
Pathogen Infection Assay performed using DC3000 and a compromised strain show that *e3l1* mutants are compromised in their ability to manifest PTI-associated resistance

Pathogen Infection Assay performed using a flg22 or mock pretreatment followed by DC3000 infection again show that *e3l1* mutants are compromised in their ability to manifest PTI-associated resistance

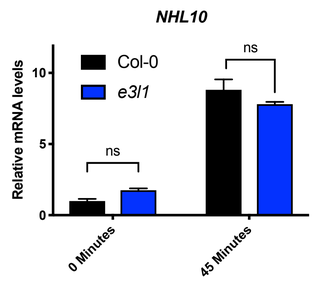
E3L1 is not required for early PTI-associated molecular phenotypes



FLS2 levels are approximately the same in *e3l1-1* plants compared to Col-0, and ligand-induced endocytosis is unaltered, indicating perception is likely not compromised in *e3l1* mutants

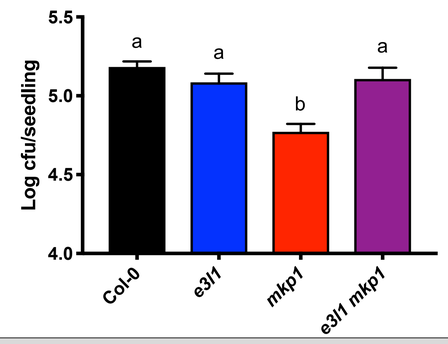


ROS Accumulation Assay after a flg22 treatment shows that *e3l1* plants are NOT compromised in inducing some PTI-associated molecular responses



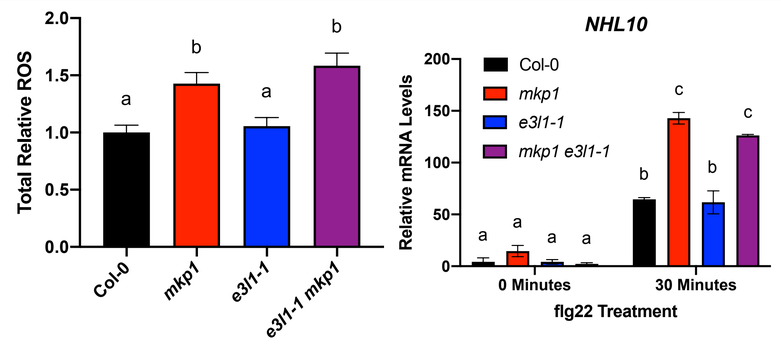
Accumulation of defense-induced transcripts again demonstrates that *e3l1* plants are NOT compromised in their ability to manifest some PTI-associated responses

Resistance in *mkp1* plants is dependent upon E3L1



Pathogen Infection Assay using DC3000 shows suppression of *mkp1*-dependent resistance in the *e3l1 mkp1* double knockout, indicating a genetic link between MKP1 and E3L1. P<0.0001.

E3L1 is not required for *mkp1*-associated molecular PTI phenotypes

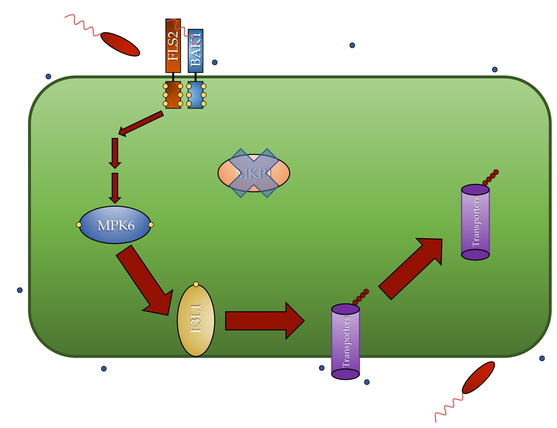


Increased ROS production shown in the *mkp1* mutant is not suppressed in the *e3l1 mkp1* double mutant. P<0.05.

Hyper-accumulation of defense related transcripts in *mkp1* plants is independent of E3L1. P<0.0001.

In *mkp1* plants, several PTI-associated molecular phenotypes are observed. Despite increased resistance being dependent on E3L1, however, these phenotypes are completely independent, indicating a specific role for E3L1 in PTI-associated resistance.

Proposed Model



In the *mkp1* mutant, removing negative regulation on MPK6 results in E3L1 hyperactivity, leading to a decrease in transporters at the plasma membrane, and therefore fewer bioactive extracellular metabolites

Future Directions

- Identify other members of the MKP1-E3L1 defense pathway
- Assay effector delivery in *e3l1 mkp1* mutants to identify if wild type levels are restored
- Identify targets of E3L1 in plant immunity

Acknowledgements

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