The role of the tail of fungal kinesin-3 in binding to early endosomes and their role in plant pathogenicity.

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Ewa Bielska

Abstract

The dimorphic fungus *Ustilago maydis* is a pathogen of maize and it was used for decades to understand the molecular basis of plant pathogenicity aspects. Recently, much effort went into understanding the cell biology that underlies the virulence of *U. maydis*. It was shown previously that early endosomes (EEs) move bidirectionally within fungal hyphal cells. Although it was shown that the motility of EEs facilitates growth of the infectious hypha and mutants defective for kinesin-3 (Kin3), the major EE transporter, exhibit impaired polarized growth, the importance of EEs and their motility in plant colonization is not known. The first part of this thesis is focused on the role of EE motility during plant infection. In collaboration with Natalie Steinberg, who performed the plant infection assays, I used a synthetic molecular anchor, K1rPX, to block the motility of EEs at early and late stages during the host plant infection and I found that EE motility is essential during the first two days of pathogenic development, when infectious hyphae exhibit most prominent elongation, whereas blockage of EE motility after 3 days post infection does not inhibit plant colonization. Moreover, I documented that the blockage of EE motility during early stages of the infection causes high plant defence response, which means that the pathogen becomes recognized by the host plant defence system. These results indicate that EE motility is crucial during initial stages of the plant host infection and enables colonization by *U. maydis* and additionally suggests involvement of EEs in some defence response machinery. The second part of the thesis addresses the relationship between Kin3, the major motor for EE motility, and the microtubule (MT) array. I demonstrate here that Kin3 uses all MT tracks available in the cell, which is in contrast to published results in other systems. In the third part I focused on the interaction between Kin3 and the EEs. I found that the pleckstrin homology (PH) domain localized at the distal part of the Kin3 tail is of minor importance for EE association. This conclusion is supported by *in vivo* experiments, showing that truncated Kin3ΔPH, which lacks the PH domain, was still able to bind to the organelles. By systematic truncation of parts of the Kin3 tail I found two adjacent regions, a DUF3694 domain and a "linker" region, that are important for binding of Kin3 to EEs. By using a synthetic anchor

composed of Kin1 rigor domain and selected Kin3 domains I proved that both domains anchor the EEs to MTs and inhibit EE motility. I also showed that the PH domain is not able to block EE motility. In collaboration with Dr. Nicholas Harmer, who performed structural modelling of selected PH domains, I demonstrated that the PH domain is likely to interact with the motor domain of Kin3. This result was confirmed by using a yeast-two hybrid approach and a protein affinity assay. This indicates a globular organization of the Kin3 motor, which was confirmed by a split-YFP assay in living cells. Deletion of the PH domain and most probably lack of intramolecular interaction between the tail and motor domain reduces Kin3 motility parameters like velocity, frequency and run length indicating that the interaction of the PH domain with the motor domain has a role in the control of Kin3 motility.

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