Evolutionarily distinct RXLR effectors from distantly related oomycetes target the plant exocyst

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Abstract
The endocytic cycle plays a crucial role in plant defence. For example, the recycling of membrane receptors has been shown to be essential for their signalling activities. It is logical therefore that plant pathogens will attempt to manipulate this process. Using yeast-2-hybrid (Y2H) analysis we have discovered that the Phytophthora infestans effector AVR3a interacts with the potato homologue of Sec5. Sec5 is a component of the multi-subunit exocyst vesicle tethering complex which is specifically involved in trafficking between Golgi and the plasma membrane. We have also found that the Arabidopsis Sec5 homologue is targeted by diverse RXLR effectors from Hyaloperonospora arabidopsidis, indicating that it is a pivotal target for oomycete pathogenicity. Moreover, we show that evolutionarily distinct RXLR effectors from either P. infestans or H. arabidopsidis interact strongly with Sec5 homologues from either potato or Arabidopsis, indicating that these effectors are functionally related and capable of similar interactions in diverse plant hosts. Using fluorescent protein tags, we localise Sec5 and Sec5-effector interactions during infection.

Introduction
The secretory pathway is vital to plant defence. It is the route by which antimicrobial proteins and other molecules are secreted. Furthermore, the endocytic recycling of membrane receptors, such as FLS2 (Robatzek et al., 2006), is thought to be an essential step in signal transduction. The secretory system is therefore an obvious target for manipulation by pathogen effectors. Oomycetes contain some of the world’s most devastating plant pathogens, including P. infestans, the cause of late blight in potato and tomato. H. arabidopsidis meanwhile provides a valuable model pathosystem allowing exploitation of the arabidopsis resources.

Results

Fluorescent protein (FP) fusions to the potato Sec5 were constructed and expressed transiently using agroinfiltration in Nicotiana benthamiana plants. The fluorescence was observed in varying proportions in the cytoplasm and small mobile bodies (Fig.3 a and b; chloroplasts imaged with their intrinsic autofluorescence are magenta or blue). The mobile bodies were of different sizes (Fig.3c). A similar localisation was seen for fluorescently tagged arabidopsis Sec5b (results not shown).

To study Avr3a-Sec5 interaction in planta, split YFP fusions were co-expressed in N. benthamiana by agroinfiltration. Despite the difficulties of the bimolecular fluorescence system we consistently obtained a clearly greater fluorescent signal from YC-Sec5 co-expressed with YN-Avr3a (Fig. 5a) compared to YC-Sec5 co-expressed with an untagged YN (Fig. 5b), which supports a specific interaction.

Future work
We are investigating the function of Sec5 using silencing in potato and T-DNA knockouts in arabidopsis. We are also assessing whether Avr3a alters secretion. Avr3a was found to interact with a second exocyst component, Sec5. Preliminary results indicate that FP-tagged Sec3 shows the same localisation as Sec5.