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

Petra BOEVINK[1], Maria Brisa RAMOS[1], Miles ARMSTRONG[1,2], Rebecca ALLEN[3], Eleanor GILROY[1], Sue DONOVAN[3], Anne REHMANY[3], Jim BEYNON[3], Paul RJ BIRCH[1,2]
[1]Plant Pathology Programme; [2]Division of Plant Science, University of Dundee, Scottish Crop Research Institute, Invergowrie, Dundee DD2 5DA, UK; [3]Warwick HRI, University of Warwick, Wellesbourne, Warwick CV35 9EF, UK.

Abstract

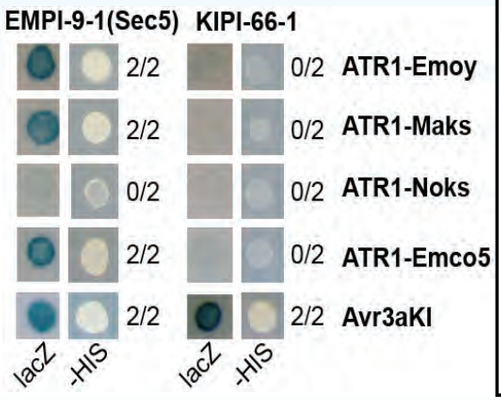
The endocytic cycle plays a crucial role in plant defence. For example, the recycling of membrane receptors has been shown to be essential for their signalling activities. It is logical therefore that plant pathogens will attempt to manipulate the endocytic process. Using yeast-2-hybrid (Y2H) analysis 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

Fig. 1



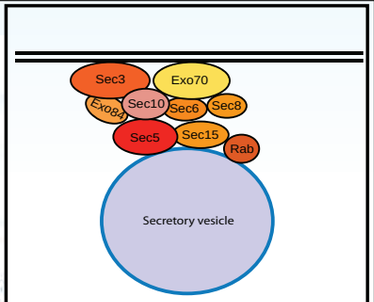
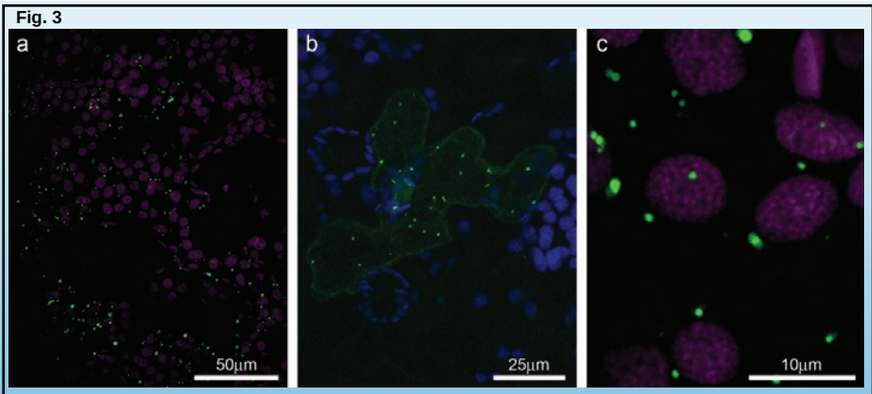


Fig. 2 A diagram of the exocyst linking a secretory vesicle to the plasma membrane (adapted from Wu *et al*. 2008)

The *P. infestans* RXLR-containing effector Avr3a, is recognised by the potato resistance protein R3a and has an essential virulence function (unpublished results). We conducted a screen with Avr3a using the Invitrogen ProQuest GAL4-based Y2H system to identify potential host target proteins. An exciting candidate from this screen was the potato homologue of the exocyst component Sec5 (Fig. 1). The exocyst is a multiprotein complex involved in secretory vesicle fusion events (see diagram in Fig. 2). An independent Y2H screen of the *H. arabidopsidis* effector ATR1 against arabidopsis and potato libraries indicated that although this effector is evolutionarily unrelated to Avr3a it nonetheless shared a host target; Sec5 (the arabidopsis Sec5b; Fig. 1). Avr3a also interacted with the arabidopsis Sec5b. The two most common alleles of *Avr3a*, K80I103 (KI) and E80M103 (EM; R3a-recognised and not recognised respectively), both interacted strongly with Sec5, whereas one of the ATR1 alleles tested, Noks, did not (Fig. 1). The ATR1 alleles did not interact with a second Avr3a interactor KIPI-66-1.

Fig. 3



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).

References
Hardham A (2007) Cell biology of plant-oomycete interactions. Cell. Microb. 9(1), 31-39
Robatzek S, Chinchilla D, Boller T (2006) Ligand-induced endocytosis of the pattern recognition receptor FLS2 in Arabidopsis. Genes Dev. 20: 537-542
Whisson SC, Boevink PC, Moleleki L, Avrova AO, Morales JG, Gilroy EM, Armstrong MR, Grouffaud S, van West P, Chapman S, Hein I, Toth IK, Pritchard L, Birch PRJ (2007) A translocation signal for delivery of oomycete effector proteins into host plant cells. Nature 450, 115-118
Wu H, Rossi G, Brennwald P (2008) The ghost in the machine: small GTPases as spatial regulators of exocytosis. Trends Cell Biol. 18(9), 397-404

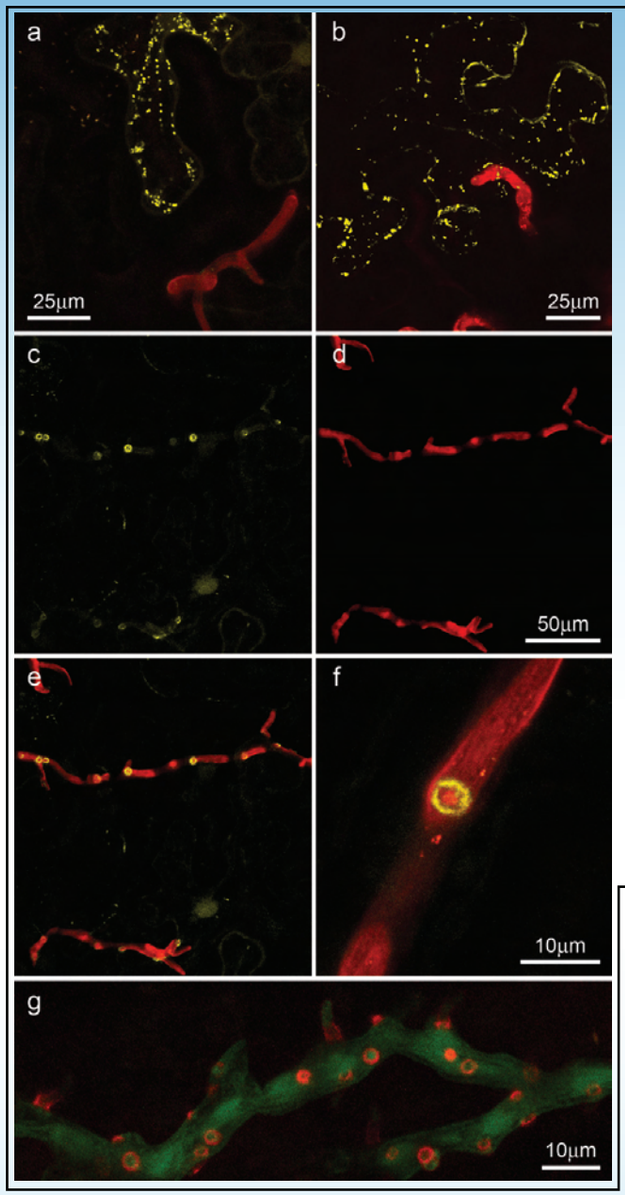
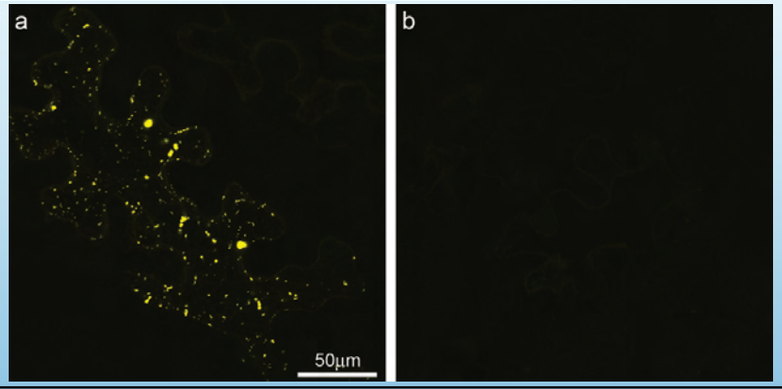


Fig. 4
N. benthamiana leaves expressing YFP-Sec5 were infected with tdTomato-tagged *P. infestans* (Fig. 4). YFP-Sec5 expressing cells not in contact with P. infestans display the same distribution of mobile objects with varying levels of cytoplasmic fluorescence (Fig. 4a). The cell in Fig. 4b is apparently in contact with a hypha but no haustorium formation was evident. Cells penetrated by haustoria (Fig. 4c-f) show an accumulation of YFP-Sec5 fluorescence around the haustoria. Note that in these images the outlines of the plant cells are not visible.

As the exocyst is an essential component of the secretory pathway and much of the secretory machinery is known to accumulate around oomycete haustoria (Hardman, 2007, references therein and personal observations) the behaviour of YFP-Sec5 suggests that the fusion maintains the correct targeting. The YFP-Sec5-labelling of haustoria appears strikingly similar to the labelling by the Avr3a-mRFP fusion expressed from transgenic *P. infestans* (Fig. 4g and Whisson et al, 2007) and thus the translocated Avr3a would be ideally located to interact with Sec5.

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.

Fig. 5



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, Sec3. Preliminary results indicate that FP-tagged Sec3 shows the same localisation as Sec5.

UNIVERSITY OF DUNDEE
THE UNIVERSITY OF WARWICK
scri living technology