

### ECFG15 ROME • ITALY 2020



Clathrin-mediated endocytosis facilitates internalization of *Magnaporthe oryzae* effectors into rice cells

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### M. oryzae effectors



*M. oryzae* cytoplasmic effectors are delivered from the BIC and internalized into the rice cell's cytoplasm by endocytosis.



Biotrophic Interfacial Complex (BIC): contains mainly cytoplasmic effectors

Oliveira-Garcia & Valent (2015) Curr Opin Microbiol. 25 : 92-101.

### The BIC organization on an invasive primary hypha



# BICs are formed by multiple vesicles containing mainly cytoplasmic effectors





### **Dynamics of BIC and cytoplasmic effector delivery**



undergoing

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### **Tagging two mechanisms of plant endocytosis**



Plant plasma membrane

Plant plasma membrane

### clathrin-mediated endocytosis (CME)

### clathrin-independent endocytosis (CIE)

#### Lti6b (Plant plasma membrane marker protein) localization





- BICs are formed by plant plasma membrane (see Lti6b:eGFP localization – green fluorescence)



#### Plant plasma membrane associated effector: Bas83



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#### Bas83 localizes at BICs, EIHM and plasma membrane of vesicles (see Bas83:mRFP localization – red fluorescence)

# **Plant Actin localization**



#### Control

#### Rice cells infected by *M. oryzae*, KV209



- BICs contain large amounts of plant Actin (see LifeAct:eGFP localization – green fluorescence)









### **Plant Clathrin localization**





- BICs contain large amounts of plant Clathrin (see CLC1:eGFP localization – green fluorescence)



### **Plant Clathrin dynamics**





### **BICs contain large amounts of plant Clathrin**



### Silencing of rice endocytosis machinery



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Biotrophic fungal infection requires mainly clathrin-mediated endocytosis.

# **Silencing of rice endocytosis**



### RNAi-Os*Flot1*

### RNAi-OsAP2



#### Biotrophic fungal infection requires mainly clathrin mediated endocytosis.



# **Chemical plant endocytosis inhibition**

	Chemical	Target	Mode of action	Comments	References
Clathrin-	Filipin	CIE*	Binds to sterols in the membrane.	Toxic at higher concentration.	(Rodal et al., 1999)
lependent	Methyl-β- cyclodextrin	CIE	Deplete cellular membranes of sterol by increasing the water solubility of the sterol.	caveolae-dependent endocytosis.	(Rodal et al., 1999)
	Chlorpromazine	CME**	Translocates clathrin and AP2 from the cell surface to intracellular endosomes.	Also inhibits CIE in some cells.	(Wang et al., 1993)
	Cantharidin	CME	PP2A (cantharidin)	Affect flg22-mediated FLS2 endocytosis.	(Serrano et al., 2007)
Clathrin- nediated	Fluazinam	CME	PP2A (cantharidin)	Affect flg22-mediated FLS2 endocytosis.	(Serrano et al., 2007)
	Triclosan	CME	PP2A (cantharidin)	Affect flg22-mediated FLS2 endocytosis.	(Serrano et al., 2007)
	Wortmannin	nd***	Inhibits PI3Ks and PI4Ks, block vacuolar trafficking, multivesicular bodies and endocytosis.	Late endosomes lose their clathrin; Potential CME association.	(Bright et al., 2001; Robinson et al., 2008; Sachse et al., 2002)
	Concanamycin A	nd	Targets V ATPase and blocks trafficking at trans- Golgi network, endosome acidification.	Induce acidification, which is crucial for many endocytic pathways.	(Maranda et al., 2001; Robinson et al., 2008)

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#### Table 1. Chemical endocytosis inhibitors

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\*CIE: clathrin-independent endocytosis; \*\*CME: clathrin-mediated endocytosis; \*\*\*nd: not determined.

# **Chemical plant endocytosis inhibition**



#### Biotrophic fungal infection requires mainly clathrin mediated endocytosis.



# **Chemical plant endocytosis inhibition**



#### Effector accumulation under appressorial pores



Biotrophic fungal infection requires mainly clathrin mediated endocytosis.

### **Conclusions**



- Based on these results we conclude that cytoplasmic effector translocation is mediated by vesicle formation, and may be characteristic of appressoria as well as biotrophic invasive hyphae.
- Clathrin-dependent endocytosis mediates internalization of *M. oryzae* effectors into rice cells
- Our results also suggest a potential role of *M. oryzae* effectors for manipulation of host cell endocytosis.

# Thank you for your attention

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