Live cell imaging of lipid droplet distribution and motility in

the filamentous fungus Ustilago maydis

Submitted by

Benjamin Roland Alexander Meadows to the University of Exeter as a thesis for the degree of Master's by Research in Biosciences September 2012

This thesis is available for Library use on the understanding that it is copyright material and that no quotation from the thesis may be published without proper acknowledgement.

I certify that all material in this thesis which is not my own work has been identified, and that no material has previously been submitted and approved for the award of a degree by this or any other University.

Abstract

Lipid droplets (LDs) are organelles specialised for lipid metabolism and storage, found across the domains of life. They are dynamic in number and size, actively transported, and have diverse functions, many of which have only recently been identified. Despite this, they remain less well-characterised than many other organelles. While the motility of LDs has been noted in filamentous fungi, no study has yet investigated its mechanism.

In this study, several techniques were established for visualisation of LDs in live cells of the dimorphic fungus *Ustilago maydis*. This species is a prominent pathogen of maize (*Zea mays*) and an established model organism for intracellular trafficking. Distribution and motility patterns of LDs were investigated quantitatively in *U. maydis* cells under varying growth conditions, including during plant infection. Active transport of LDs was found to be microtubule-dependent, and dependent on specific motor proteins and organelle interactions.

Table of contents

Ab	Abstract	
Table of contents		
List of tables		
List of figures		
Acknowledgements		
Ab	breviations	8
1. In	troduction	9
1.1	Lipid droplets	9
1.2	History of the study of lipid droplets	9
1.3	Lipid droplet structure and function	11
1.4	Lipid droplets are highly conserved structures	14
1.5	Role in human disease	15
1.6	Economic importance	15
1.7	Biogenesis and dynamics	16
1.8	Active transport	17
1.9	Role in fungal pathogenicity	18
1.10	The dimorphic pathogenic fungus <i>Ustilago maydis</i>	18
1.11	Summary	20
2. Results		21
2.1	Bioinformatics survey	21
2.2	Visualisation of lipid droplets	25
2.3	Lipid droplet intracellular distribution	32
2.4	Lipid droplet distribution during plant infection	35
2.5	Lipid droplet motility is microtubule-dependent	38
2.6	Lipid droplets comigrate with motor proteins and organelles	45
3. Discussion		52
3.1	Bioinformatics	52
3.2	Distribution	53
3.3	Motility	54
4. Co	nclusion	57
5. M	5. Methods	
5.1	Strains	58
5.2	Plasmid construction	59

5.3	Growth conditions	60
5.4	Fluorescent staining	61
5.5	Inhibitor treatment	61
5.6	Plant infection	62
5.7	Laser epifluorescence microscopy	
5.8	Data analysis	63
6. Re	64	
7. Aj		
7.1	Appendix 1: Plasmid maps	72