

TABLE OF CONTENTS

INTRODUCTION	1
1. Gibberellin signalling: DELLA proteins as master regulators	3
2. Post-translational modifications of DELLA proteins.....	5
3. Molecular mechanisms of DELLA action	8
4. References	12
OBJECTIVES	17
CHAPTER 1	21
0. Abstract	22
1. Introduction.....	23
2. Results	24
Warm temperature or shade decrease the abundance of a GA-insensitive DELLA protein.....	24
COP1 affects RGA levels in response to shade and warmth.....	24
Changes in RGA abundance precede changes in GA	26
Relative contribution of each pathway	26
COP1 promotes degradation of a GA-insensitive DELLA protein.....	27
COP1 interacts physically with GAI and RGA in yeast	27
COP1 interacts with GAI and RGA in planta.....	29
COP1/SPA1 forms a ternary complex with DELLA.....	29
COP1 ubiquitinates GAI and RGA <i>in vitro</i>	31
COP1 controls hypocotyl elongation in a DELLA-dependent manner	31
3. Discussion	34
4. Material and methods	35
Plant material.....	35
Confocal microscopy in Arabidopsis	36
Western analysis of GFP-RGA and GFP-(rga- Δ 17).....	37
Real-time qPCR	37
Gibberellin quantification	37
Degradation assays in <i>Nicotiana benthamiana</i>	38
Yeast two-hybrid assays	39
Co-immunoprecipitation.....	39
Co-localization assays in <i>N.benthamiana</i>	40
Bimolecular fluorescence complementation	40

Co-localization assays in mammalian cells	41
Analysis of confocal images of mammalian cells	41
Pull-down assays with p62-beads	42
<i>In vitro</i> ubiquitination assay	42
Growth assays	42
Acknowledgements	43
5. Supplemental information	44
6. References	50
CHAPTER 2	55
0. Abstract	56
1. Introduction	57
2. Results	58
DELLAs interact with the Paf1c subunit ELF7	58
DELLAs are part of a gene regulatory network with Paf1c	60
Both DELLAs and ELF7 influence H2Bub general abundance and distribution..	63
DELLAs are required for the recruitment of Paf1c to the chromatin	65
RNAPII occupancy and distribution is dependent on DELLAs and ELF7	67
3. Discussion	69
4. Material and methods	72
Plant material	72
Growth conditions and treatments	73
Yeast two-hybrid assays	73
Bimolecular fluorescence complementation assay	74
Co-immunoprecipitation assays	74
Gene expression analysis	74
ChIP experiments	75
ChIP-seq bioinformatics	76
Subcellular fractionation	77
Immunoblot analysis	78
Acknowledgements	78
5. Supplemental information	79
6. References	90
GENERAL DISCUSSION	95
1. Relative importance of the different mechanisms	98
2. Evolutionary implications	99

3. Biotechnological implications	101
4. References	103
CONCLUSIONS	107