The Biochemistry of GIGANTEA: A Circadian Clock-Controlled Regulator of Photoperiodic Flowering in Plants

Moyra Maria Black

A thesis submitted in fulfilment of the requirements for the degree of Doctor of Philosophy in Biological Sciences

The University of Auckland

February 2007

ABSTRACT

In many organisms, important biological and developmental processes such as growth and reproduction occur in time with particular seasons of the year. Many species recognise seasonal changes through the measurement of changes in day length (photoperiodism) by comparison to an internal oscillator called the circadian clock. Genes involved in photoperiodic processes such as flowering can be studied using the model plant *Arabidopsis thaliana (Arabidopsis)*. One *Arabidopsis* gene central to photoperiodism is *GIGANTEA* (*GI*). *GI* mutants show a delay in flowering in inductive day lengths, leading to a longer phase of vegetative growth. Expression of *GI* is regulated by the circadian clock and is cyclical, peaking in the evening. The GI protein is large, plant-specific, nuclear localised, and has no homology to any other proteins. GI accumulates during the day and drops at night due to predicted proteosomal degradation. While it has been forty years since the first mutant alleles of *GI* were described much is still unknown about the molecular mechanism of GI action.

The objective of this work was to characterise the GI protein, to give a greater understanding of the role of GI in floral induction. Soluble affinity-tagged full-length GI was expressed in *Escherichia coli* (*E. coli*) and was stabilised by the addition of the detergent N-dodecyl-β-D-maltoside (DDM) to storage and purification buffers. Stabilised GI was purified using a variety of chromatographic methods, and characterised using a selection of biochemical techniques including circular dichroism, and dynamic light scattering. This showed that purified GI contained secondary structure, but was polydisperse in solution. Limited proteolytic digests and mass spectrometry were used to identify possible GI domains. This led to the identification of a predicted 46 kDa amino terminal GI domain.

A variety of potential GI domains were targeted using either a sequence-based bioinformatics approach or an experimentally-derived approach, and were expressed in *E. coli*. While most of these domains remained intractably insoluble, the 46 kDa amino-terminal domain showed partial solubility when expressed at 10°C and lysed in buffer containing DDM. This domain was expressed from two plant species (*Arabidopsis* and Rye Grass) and large-scale expression and purification protocols developed. *In vivo* characterisation of the amino terminal GI domain in *Arabidopsis* plants suggested that while the protein product of the domain may be unstable, a region of the domain could have a dominant negative effect on flowering time in wild type plants.

Arabidopsis plants were also used to characterise the light responses of full-length GI protein. This showed that GI protein accumulated to high levels in plants exposed to blue, white and red light, with blue-light plants showing the highest levels of GI accumulation. GI protein was then shown to accumulate less in plants lacking the *CRY1* and *CRY2* blue light photoreceptors than plants with functional *CRY1*and *CRY2* genes.

ACKNOWLEDGEMENTS

Firstly, I wish to thank my supervisors Vic Arcus and Jo Putterill, for their support and guidance throughout my PhD studies. Their constant enthusiasm and curiosity, and their academic vigour have set an example that I can only hope to live up to.

Thank you to the members of the Laboratory of Structural Biology, the Agresearch Structural Biology Laboratory and the *Arabidopsis* Laboratory, for their assistance, advice and friendship over the last three and a half years. In particular, I would like to thank Karine David, who spent many patient hours teaching me about working with *Arabidopsis*, who developed many of the protocols for analysis of the transgenic plants, and who was the pioneer for much of the work performed as part of my research. Thanks also to John Steemson, who ran our lab so well, and helped me with more little things than I can ever recount, or could ever repay. I am also indebted to Nga Tama, who did a great job looking after the greenhouses, looked after my plants for me whenever I was away, and who was always there for advice on the care of my plants. Thanks also to Markus Günl for help with real-time PCR, and to Nikki Moreland for proof-reading this thesis and helping me improve my writing.

I am also grateful to Nigel Birch and the members of the Birch lab for their advice and assistance with western blots, and for letting me use their Fuji imager, to Alok Mitra for helping with the electron microscopy, and to Jackie Aitkin for help with CD spectroscopy. Thanks to Frances Ikin, who did a summer studentship with us in the Agresearch Structural Biology Lab, and worked with me on the Rye Grass H19 domain, to Milan Gagic for kindly providing me with the Rye Grass GI plasmid DNA, and to Robert Willows for providing me with the DnaK plasmid constructs. Thank you also to AGMARDT (The Agricultural and Marketing Research and Development Trust), AgResearch, and The University of Auckland, for funding me during my studies.

Finally, I would like to give a special thank you to my family, without whom I would never have made it this far. Thanks for all your love and support, and for always believing in me. Thanks especially to Geoff for being my rock, for loving me and feeding me, and making me laugh. I could not have done this without you.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABBREVIATIONS

α-CHC	α cyano-4-hydroxycinnamic acid
ACN	acetonitrile
AGL20	*AGAMOUS-LIKE 20*
Agrobacterium	*Agrobacterium tumefaciens*
AMP	ampicillin
AP1	*APETALA1*
APX	ascorbate peroxidase
Arabidopsis	*Arabidopsis thaliana*
BLAST	basic local alignment search tool
bp	base pairs
BSA	bovine serum albumin
CAB	CHLOROPHYLL A/B BINDING
CAL	*CAULIFLOWER*
CAM	chloramphenicol
CaMV	cauliflower mosaic virus
CBP	calmodulin binding protein
CCA1	*CIRCADIAN CLOCK ASSOCIATED 1*
CCR2	COLD-CIRCADIAN RHYTHM-RNA BINDING 2
CCT	CO, CO-like, TOC1
CD	circular dichroism
CDF1	*CYCLING DOF FACTOR 1*
cDNA	complementary deoxy-ribonucleic acid
CMC	critical micellular concentration
CO	*CONSTANS*
CRY1 and *2*	*CRYPTOCHROME 1* and *2*
C_T	threshold cycle
CV	column volume
CYC	cyclohexamide
DBC	deoxy-BIGCHAP
DDM	N-dodecyl-β-D-maltoside
DEX	dexamethasone
DLS	dynamic light scattering
DNA	deoxy-ribonucleic acid
dNTP	deoxy-nucleotide triphosphate
DTT	dithiothreitol
ECL	enhanced chemi-luminescence
E. coli	*Escherichia coli*
EDTA	ethylene diamine tetraacetic acid
ELF3 and *4*	*EARLY FLOWERING 3* and *4*
EM	electron microscopy
EST	expressed sequence tag
FKF1	*FLAVIN-BINDING KELCH-REPEATS, F-BOX 1*
FLC	*FLOWERING LOCUS C*
FT	*FLOWERING LOCUS T*
FUL	*FRUITFUL*
GA	gibberellin
GAPDH	glyceraldehyde-3-phosphate dehydrogenase
GENT	gentamycin
GI	*GIGANTEA*
GR	glucocorticoid receptor
GSG	oxidised glutathione
GSSH	reduced glutathione
GST	glutathione S-transferase
HEPES	N-2-hydroxyethylpiperazine-N'-2-ethanesulphonic acid
HRP	horse radish peroxidase
IEX	ion exchange
IMAC	immobilised metal affinity chromatography

IPTG	isopropyl β-D-thiogalactopyranoside
KAN	kanamycin
kb	kilobase pairs
kDa	kiloDaltons
LB	luria broth
LD	long days
LED	light emiting diode
LFY	*LEAFY*
LHY	*LATE ELONGATED HYPOCOTYL*
LOV	light, oxygen, voltage
MALDI-TOF	matrix assisted laser desorption / ionisation time of flight
MCS	multiple cloning site
MES	2-(n-morpholino)-ethanesulfonic acid
MOWSE	molecular weight search
mRNA	messenger ribonucleic acid
MS	mass spectroscopy
MW	molecular weight
NP40	nonidet P-40
NPH1	*NONPHOTOTROPIC HYPOCOTYL 1*
ocs	octopine synthase
OGT	O-linked-N-acetylglucosamine transferase
PAGE	polyacrylamide gel electrophoresis
PCR	polymerase chain reaction
PDB	protein data bank
PEG	polyethylene glycol
PHYA and *B*	*PHYTOCHROME A* and *B*
pI	isoelectric point
PIF3	Phytochrome-interacting Factor 3
ProtA	Protein A
psi	pounds per square inch
PVDF	poly (vinylidene fluoride)
RIF	rifampicin
RNA	ribonucleic acid
RNase	ribonuclease A
rpm	revolutions per minute
RT	room temperature
rTEV	recombinant tobacco etch virus
SAM	shoot apical meristem
SD	short days
SDS	sodium dodecyl sulphate
SOC1	*SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1*
SOD	superoxide dismutase
SPEC	spectinomycin
SPY	*SPINDLY*
TAP	tandem affinity purification
TB	tuberculosis
TEMED	N,N,N,N-Tetramethylethylenediamine
TFA	trifluoro acetic acid
TFL1 and *2*	*TERMINAL FLOWER 1* and *2*
TIM	timentin
TOC1	*TIMING OF CAB EXPRESSION 1*
TPR	tetratricopeptide
Tris	tris(hydroxymethyl)aminomethane
tRNA	transfer ribonucleic acid
UV	ultraviolet
WT	wild type
X-gal	5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside
ZT	zeitgeber time
ZTL	*ZEITLUPE*

The Flower

Once in a golden hour
I cast to earth a seed.
Up there came a flower,
The people said, a weed.

To and fro they went
Thro' my garden bower,
And muttering discontent
Cursed me and my flower.

Then it grew so tall
It wore a crown of light,
But thieves from o'er the wall
Stole the seed by night.

Sow'd it far and wide
By every town and tower,
Till all the people cried,
"Splendid is the flower!"

Read my little fable:
He that runs may read.
Most can raise the flowers now,
For all have got the seed.

And some are pretty enough,
And some are poor indeed;
And now again the people
Call it but a weed.

Alfred, Lord Tennyson