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

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degree of Doctor of Philosophy in Biological Sciences**

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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.

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ABBREVIATIONS

α -CHC	α cyano-4-hydroxycinnamic acid
ACN	acetonitrile
AGL20	AGAMOUS-LIKE 20
<i>Agrobacterium</i>	<i>Agrobacterium tumefaciens</i>
AMP	ampicillin
AP1	APETALA1
APX	ascorbate peroxidase
<i>Arabidopsis</i>	<i>Arabidopsis thaliana</i>
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 micellar 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
<i>E. coli</i>	<i>Escherichia coli</i>
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 emitting diode
LFY	<i>LEAFY</i>
LHY	<i>LATE ELONGATED HYPOCOTYL</i>
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
<i>NPH1</i>	<i>NONPHOTOTROPIC HYPOCOTYL 1</i>
ocs	octopine synthase
OGT	O-linked-N-acetylglucosamine transferase
PAGE	polyacrylamide gel electrophoresis
PCR	polymerase chain reaction
PDB	protein data bank
PEG	polyethylene glycol
<i>PHYA</i> and <i>B</i>	<i>PHYTOCHROME A</i> and <i>B</i>
pl	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
<i>SOC1</i>	<i>SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1</i>
SOD	superoxide dismutase
SPEC	spectinomycin
<i>SPY</i>	<i>SPINDLY</i>
TAP	tandem affinity purification
TB	tuberculosis
TEMED	N,N,N,N-Tetramethylethylenediamine
TFA	trifluoro acetic acid
<i>TFL1</i> and <i>2</i>	<i>TERMINAL FLOWER 1</i> and <i>2</i>
TIM	timentin
<i>TOC1</i>	<i>TIMING OF CAB EXPRESSION 1</i>
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	<i>ZEITLUPE</i>

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