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**Characterising GIGANTEA interactors: the
Arabidopsis BELL-LIKE HOMEODOMAIN 3 and
BELL-LIKE HOMEODOMAIN 10 proteins**

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ABSTRACT

The ability to detect and respond to environmental signals is fundamental in coordinating floral induction in plants to favourable conditions. An important flowering time cue is day length and it is proposed that light signals are perceived and measured by an interaction between photoreceptors and an internal pacemaker, the circadian clock. The control of flowering has been best characterised in the model plant *Arabidopsis thaliana* L. Heynh (*Arabidopsis*). The *GIGANTEA* (*GI*) gene has a complex role in both the promotion of flowering in response to photoperiod and the regulation of the circadian clock. The expression of *GI* is under circadian control and is affected by day length, light quality and temperature changes. The *GI* protein is also circadian regulated and is actively degraded in the dark.

The biochemical function of *GI* is unknown and one method to elucidate the role of this protein is to identify protein interactors. The aim of this thesis project was to characterise proteins that interacted with *GI*. Previously, the BELL-LIKE HOMEODOMAIN 3 (*BLH3*) protein was identified as a putative *GI* protein interactor. As part of this thesis work, yeast 2-hybrid and *in vitro* pull down assays were utilised to confirm the interaction between *GI* and *BLH3*. Sequence and phylogenetic analyses were used to further examine the BELL family of proteins. The BELL-LIKE HOMEODOMAIN 10 (*BLH10*) protein was found to be closely related to *BLH3* and also interacted with *GI*. Reverse 2-hybrid assays were used to determine the regions or domains within the *GI*, *BLH3* and *BLH10* proteins required to mediate protein interactions.

Expression assays established that the *BLH3* and *BLH10* transcripts were present throughout plant tissues and times of development. Further analyses revealed that *BLH3* and *BLH10* are not directly regulated by the circadian clock. The results of GFP expression assays demonstrated that the *BLH3* protein is localised to the nucleus in plant cells. Transgenic *blh3* and *blh10* mutant plants were identified and analysed for flowering and light response phenotypes. *BLH3* and *BLH10* do not function with *GI* in the photoperiodic pathway to control flowering, yet the *blh3* and *blh10* mutants do have a flowering phenotype in short day conditions. Like *gi*, the *blh3* and *blh10* mutants exhibited exaggerated hypocotyl elongation in response to red and low light conditions. These results are suggestive of a role for *BLH3*, *BLH10* and *GI* in flowering and de-etiolation responses to specific light conditions in plants.

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ABBREVIATIONS

A	adenine
aa	amino acid
Ac	acetate
AD	transcription activation domain
ATP	adenosine triphosphate
BD	DNA binding domain
bHLH	basic/helix-loop-helix class of transcription factors
BIS	N,N'-Methylene-bis-acrylamide
BLAST	basic local alignment search tool
bp	base pair(s)
Bq/MBq	Bequerels, megaBequerels
C	cytosine
CaMV 35S	cauliflower mosaic virus 35S promoter sequence
cDNA	complementary DNA
cM	centimorgan(s)
Col-0	<i>Arabidopsis thaliana</i> ecotype Columbia
CTAB	cetyl-trimethylammonium bromide
C-terminus	carboxyl terminus
CVI	<i>Arabidopsis thaliana</i> ecotype Cape Verde Islands
d	day(s)
Da, kDa	dalton, kilodalton
DAPI	4',6-diamidino-2-phenylindole
dCTP	2-deoxycytidine-5-triphosphate
D	dark
DD	continuous dark conditions
dex	dexamethasone
DMDC	dimethyl-dicarbonate
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
DNase	deoxyribonuclease

dNTP	deoxynucleotide triphosphate
DTT	1,4 - dithiothreitol
dT	deoxythymidine
EDTA	ethylene diamine tetraacetic acid
EMS	ethyl methylsulfonate
EST	Expressed Sequence Tag
EtBr	ethidium bromide
FR	far-red light
g, µg, ng, pg	grams, micrograms, nanograms, picograms
G	guanine
GAL4	<i>Sacromyces cervisiae</i> GAL4 transcription factor
GFP	green fluorescent protein
GR	glucocorticoid receptor
GUS	<i>E. coli</i> β-glucuronidase reporter gene
h	hour
HA	Influenza A haemagglutinin protein epitope
HD	homeodomain region
HEPES	N-[2-hydroxyethyl] piperazine-N'-[2-ethanesulfonic acid]
IM	infiltration media
IPTG	isopropylthio-β-D-thiogalactoside
kb	kilobase(s)
L, mL, µL	litre, millilitre, microlitre
LB	left T-DNA border
LD	long day conditions (18 h light; 6 h dark)
Ler	<i>Arabidopsis thaliana</i> ecotype Landsberg erecta
leu	leucine
L	light
LL	continuous light conditions
m, cm, nm	metre, centimetre, nanometre
MBP	Maltose Binding Protein
min	minute
mRNA	messenger RNA
miRNA	microRNA
M, mM, µM	moles per litre, millimoles per litre, micromoles per litre

MOPS	3-[N-morpholino]propanesulfonic acid
MW	molecular weight
mya	million years ago
NCBI	National Centre for Biotechnology Information
<i>nptII</i>	<i>neomycin phosphotransferase II</i> gene
<i>ocs</i> 3'	<i>octopine synthase</i> 3' terminator sequence
OD	optical density
Pa, kPa	Pascal, kiloPascal
PBS	phosphate buffered saline
PEG ₄₀₀₀	polyethylene glycol, MW 4000
pers. comm.	personal communication
pfu	plaque forming unit
<i>pnos</i>	<i>nopaline synthase</i> promoter region
psi	pounds per square inch
QTL	quantitative trait loci
RB	right T-DNA border
Re	constant red light conditions
RNA	ribonucleic acid
RNase	ribonuclease
rRNA	ribosomal RNA
s	second
SD	short day conditions (8 h light; 16 h dark)
SDS	sodium dodecyl sulfate
SEM	standard error of the mean
SSC	standard saline citrate
T	thymine
TAE	tris-acetate-EDTA
<i>Taq</i>	<i>Thermus aquaticus</i>
TBE	tris-borate-EDTA
TE	tris-EDTA
TEMED	N,N,N',N'-Tetramethyl-ethylendiamine
Tris	tris(hydroxymethyl)-aminomethane
trp	tryptophan
T-DNA	transfer DNA

U	unit
UTR	untranslated region
UV	ultra violet light
V	volt(s)
v/v	volume/volume
w/v	weight/volume
×g	times the force of gravity
~	approximate
#	number
°C	degrees Celsius
95% CI	95% confidence intervals

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