# 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 | *Arabidopsis thaliana* ecotype Columbia |
| CTAB | cetyl-trimethylammonium bromide |
| C-terminus | carboxyl terminus |
| CVI | *Arabidopsis thaliana* 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 | *Sacromyces cerivisiae GAL4* transcription factor |
| GFP | green fluorescent protein |
| GR | glucocorticoid receptor |
| *GUS* | *E. coli ß-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-ß-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 | *Arabidopsis thaliana* 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 |
| *nptII* | *neomycin phosphotransferase II* gene |
| *ocs* 3' | *octopine synthase* 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 |
| *pnos* | *nopaline synthase* promoter region |
| psi | pounds per square inch |
| QTL | quantitative trait loci |
| RB | right T-DNA border |
| Rc | 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 |
| *Taq* | *Thermus aquaticus* |
| 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 |
| $^{o}$C | degrees Celsius |
| 95% CI | 95% confidence intervals |

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