Functional interactions between TALE and lateral organ boundary transcription factors in regulation of flowering in *Arabidopsis thaliana*

By

BRENDA SUNTWE CHISANGA SALASINI

A thesis submitted to the Faculty of Graduate and Postdoctoral Affairs in partial fulfillment of the requirements for the degree of

Master of Science
In
Biology

Carleton University
Ottawa, Ontario, Canada

ABSTRACT

In Arabidopsis (*Arabidopsis thaliana*), floral inductive signals acting on the shoot apical meristem (SAM) cause acquisition of inflorescence meristem (IM) fate. This process depends on PENNYWISE (PNY) and POUND-FOOLISH (PNF). These factors maintain meristem activity essential for flowering by repressing a lateral organ boundary module comprising *BLADE-ON-PETIOLE1/2* (*BOP1/2)* and its downstream effectors: *ARABIDOPSIS THALIANA HOMEOBOX GENE1* (*ATH1*) and *KNOTTED1-LIKE FROM ARABIDOPSIS THALIANA6 (KNAT6)*. Misexpression of this module in *pny pnf* apices abolishes flowering but the mechanism was unclear. BOP1/2 are BTB-ankryin transcriptional co-activators that require TGACG motif-binding (TGA) basic leucine zipper (bZIP) proteins for recruitment to DNA. A steroid induction system was used to show that BOP1 directly activates *ATH1* but binding sites were unknown. My data pinpoint the location of bZIP-binding elements in a segment of the *ATH1* promoter that shows responsiveness to BOP1 induction and provide genetic evidence to show that clade I TGA factors are part of this pathway. I also provide evidence that this module perturbs the cross-talk between gibberellin (GA) and jasmonic acid (JA) pathways to abolish flowering in *pny pnf* mutants. Collectively, these data shed light on the role of lateral organ boundary genes in flowering.

ACKNOWLEDGEMENTS

All to the glory of GOD, the supreme and the almighty

Foremost, I would like to express my sincere gratitude to my advisor Dr. Shelley Hepworth for her continuous support of my Master's degree, for her patience, motivation, and immense knowledge. Her guidance saw me through in all the time of my research and writing of this thesis. I could not have imagined having a better advisor and mentor for my study.

My sincere gratitude also goes to my advisory committee members Dr. John Vierula and Dr. Maria-Andree Akimenko for their guidance and to Dr. Owen Rowland for enlightening me with the awesome power of genetics "APOG" through his genetics course.

I am also grateful to my fellow lab mates in the Hepworth and Rowland labs, including Bhaswati Devi, Natalie Woerlen, John Lock, Dr. Madiha Khan, Nayana de Silva and Dr. Jhadeswar Murmu for stimulating interesting ideas and discussions, and for the sleepless nights we were working together before deadlines, and for all the fun we have had during my studies. Also I thank my friends Ahllam, Kamya, and Gigi for their constant emotional support.

My sincere and heart-felt gratitude to Professor Douglas Anglin for sponsoring my Master's study and to Bondunde Oyetoran, her family, and friends for the precious emotional support and the much needed help in every walk of my stay during these past years of graduate study.

Last but not least, I would like to thank my family: my parents Stevenia Katongo and Bernard Chisanga for their love and supporting me spiritually throughout my life. I owe my deepest gratitude to my husband, John Salasini and our children Matampulo III and Wila for their love, patience and understanding. I am blessed to have these wonderful people in my life.

PREFACE

This thesis explores the mechanism by which *BLADE-ON-PETIOLE* genes function as transcription factors in modulating meristem identity and flowering. Submitted publications containing figures and text from this thesis include:

Repression of lateral organ boundary genes by PENNYWISE and POUND-FOOLISH is essential for meristem maintenance and flowering in *Arabidopsis thaliana* (2015) Madiha Khan, Laura Ragni, Paul Tabb, Brenda C. Salasini, Steven Chatfield, Raju Datla, John Lock, Xiahezi Kuai, Charles Després, Marcel Proveniers, Cao Yongguo, Daoquan Xiang, Halima Morin, Sylvie Citerne, Shelley R. Hepworth*, and Véronique Pautot* (Plant Physiology, under review).

I carried out the majority of work in this thesis but would like to thank the following individuals for their important contributions:

Dr. Madiha Khan and Paul Tabb initiated an investigation of the *pny pnf* mutant showing that inactivation of *BOP1/2*, *KNAT6*, and *ATH1* rescues flowering. Madiha further identified that BOP1 directly activates *ATH1* which was the starting point for my project. Madiha also provided RT-PCR data in characterizing the different *ath1* alleles, created a map of the *ATH1* promoter, and created the *ATH1p:GUS D35S:BOP1-GR* lines for promoter analysis. John Lock assisted me in functional characterization of the *ATH1* promoter as part of his BIOL 4908 undergraduate thesis research project. Xiahezi Kuai and Charles Després at Brock University provided the ChIP data presented in Figure 3.6.

Constructs for Bimolecular Fluorescence Complementation were generated by Natalie Woerlen. Bhaswati Devi taught me the infiltration technique. Denise Chabot and Keith Hubbard from Agriculture and Agri-Food Canada assisted me in collecting images using confocal laser scanning fluorescence microscopy.

My supervisor, Dr. Shelley Hepworth, generated the crosses between *pny* and *pny pnf* with *tga1* and *tga4*, analyzed the microarray data, and assisted in the characterization of *D35S:ATH1* and *bop1-6D aos* lines. I would also like to thank Dr. Hepworth for assistance in making some of the figures and editing this thesis.

TABLE OF CONTENTS

GLOSSARY OF GENETIC TERMS

Loss-of-function: loss or reduction of activity

Gain-of-function: ectopic or increased activity

Phenotypic suppression: shift towards wild-type phenotype

Phenotypic enhancement: worsening a mutant phenotype

Redundancy: when there is genetic compensation in the event of gene loss-of-function

Homolog: genes sharing a common ancestor in evolution

Ortholog: genes in different organisms that descend from a common ancestor (often with the same function)

Paralog: genes that are related by a duplication event

GENETIC NOMENCLATURE IN *ARABIDOPSIS THALIANA*

Acropetal: Development of organs from the base upward towards the shoot apex

Basipetal: Development of organs from the shoot apex downward toward the base

Wild type gene: *BOP1*

Wild type protein: BOP1

Loss-of-function mutant (homozygous): *bop1*

Loss-of-function mutant (heterozygous): *bop1/+*

Gain-of-function mutant (dominant): *bop1-6D*

Double mutation: *bop1 bop2*

Promoter fusion to a gene coding region*: 35S:BOP1*

Protein fusion: BOP1-GR

LIST OF ABBREVIATIONS

AP1	*APETELA1*
ATH1	*ARABIDOPSIS THALIANA HOMEOBOX GENE1*
BELL	*BEL1-LIKE*
BiFC	Bimolecular Fluorescence Complementation
BOP	BLADE-ON-PETIOLE
BTB/POZ	Bric-a-Brac, Tram Track, Broad Complex/POX virus and Zinc finger
bZIP	basic leucine zipper
CLV	*CLAVATA*
CO	*CONSTANS*
Col	Columbia (wild-type ecotype of *Arabidopsis thaliana*)
CUC	*CUP-SHAPED COTYLEDONS*
CZ	Central zone
D35S CaMV	Double 35S Cauliflower Mosaic virus promoter
DEX	Dexamethasone
FLC	*FLOWERING LOCUS C*
FT	*FLOWERING LOCUS T*
GA	Gibberellin
GR	Glucocorticoid receptor
GUS	β-Glucuronidase
IM	Inflorescence meristem
KNAT	*KNOTTED-like FROM ARABIDOPSIS THALIANA*

KNOX	*KNOTTED1-like HOMEOBOX*
LFY	*LEAFY*
LD	Long days
MeJA	Methyl Jasmonate
miR156	miRNA156
NPR1	*NONEXPRESSOR OF PATHOGENESIS RELATED GENES1*
OC	Organizing center
PAN	PERIANTHIA
PCR	Polymerase chain reaction
PNF	*POUNDFOOLISH*
PNY	*PENNYWISE*
PR	*PATHOGENESIS RELATED*
PZ	Peripheral zone
qRT-PCR	Quantitative reverse transcriptase polymerase chain reaction
RIM	Resistance-induced by methyl jasmonate
RZ	Rib zone
SA	Salicylic acid
SAM	Shoot apical meristem
SD	Short days
SOC1	*SUPRESSOR OF OVEREXPRESSION OF CONSTANS1*
SPL	*SQUAMOSA PROMOTER BINDING PROTEIN-like*
STM	*SHOOT MERISTEMLESS*

TALE	THREE-AMINO ACID-LOOP-EXTENSION
TFL1	*TERMINAL FLOWER1*
TGA	TGACG-motif binding
T-DNA	Transfer DNA
WUS	*WUSCHEL*
YFP	Yellow fluorescent protein

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

INTRODUCTION

1.1 Thesis overview

The transition from vegetative to reproductive development is an important decision in the life cycle of a plant. Internal and external signals acting on the shoot apical meristem (SAM) cause its restructuring to form an inflorescence meristem (IM) (Bernier, 1988). This results in new patterns of aerial development, seen as the transition from making leaves to the production of an inflorescence with flowers separated by internodes. Two THREE-AMINO-ACID-LOOP-EXTENSION (TALE) homeodomain proteins called PENNYWISE (PNY) and POUND-FOOLISH (PNF) facilitate this transition (Smith et al., 2004; Hamant and Pautot, 2010). In *pny pnf* mutants, shoot meristems support the production of leaves but internode elongation and flower formation is blocked (Smith et al., 2004). This block is due to the misexpression of a set of lateral organ boundary genes including *BLADE-ON-PETIOLE1/2* and downstream factors *ARABIDOPSIS THALIANA HOMEOBOX GENE1* (*ATH1*) and *KNOTTED1-LIKE FROM ARABIDOPSIS THALIANA6* (*KNAT6*) (Tabb, 2012). *BOP1/2* promote *ATH1* and *KNAT6* expression and the three form a module that antagonizes meristem competence to flower (Khan et al., 2015).

My thesis sheds light on regulatory interactions that define this module and block meristem competence to flower. BOP1/2 are members of a BTB-ankyrin family of transcription factors that lack a DNA binding domain and require TGACG motif-binding (TGA) basic leucine zipper (bZIP) proteins for recruitment to DNA. In the first part of my thesis, I define interaction sites for BOP1 on the *ATH1* promoter. In the second part of my thesis, I provide evidence that clade I TGA factors mediate BOP1/2 function. In the third part of my thesis, I used transcriptome profiling to form the hypothesis that BOP1/2 block

flowering by promotion of jasmonate defense pathways. I tested whether this takes place through repression of gibberellin signals for flowering. Understanding how the BOP1/2 module represses IM activity provides insight into the control of plant development and reproduction.

1.2 *Arabidopsis thaliana* is a model plant species

Arabidopsis thaliana or mouse ear cress was widely adopted as a genetic model for plants in the 1980's based on its short life cycle, ease of growth, simple genetics, and numerous progeny (Meyerowitz, 1989; Somerville and Koorneef, 2002). Before this time, popular genetic models included commercial crops such as maize, barley, tomato, and petunia (Serino and Guiliana, 2011). Whilst studies in these species advanced our early understanding of plant development and provided a foundation for discovery of novel traits for crop improvement, these models were disadvantaged by their large size and long generation time posing a challenge for research. To address some of these drawbacks, the small crucifer species *Arabidopsis thaliana* (Arabidopsis) was developed as a genetic model for plant studies (Meyerowitz, 1989; Somerville and Koorneef, 2002).

Arabidopsis is a small herbaceous weed in the mustard family. Unlike many plants, this species is a self-fertilizing diploid with prolific seed production. The Arabidopsis genome was fully sequenced in 2000 revealing that its five chromosomes contain about 27,000 protein-coding genes on par with other eukaryotic models. Arabidopsis plants are readily transformable using Agrobacterium and amenable to common strategies for mutagenesis which is ideal for forward or reverse genetic screens (Clough and Bent, 1998; Serino and

Guiliana, 2011). Extensive community resources are available at little or no cost including a searchable database for genomic information, T-DNA and transposon insertion collections, cDNA libraries, and seed stocks accessible through The Arabidopsis Information Resource (TAIR, http://www.arabidopsis.org) and Arabidopsis Resource Center (ABRC, http://abrc.osu.edu).

Knowledge gained from studies in Arabidopsis serves as a foundation for applied work in commercially important crop species including monocots and dicots (Bevan and Walsh, 2005; Ferrier et al., 2011). Developmental programmes including those underlying the regulation of SAM maintenance and flowering are highly conserved among flowering plants. Pinpointing orthologous genes of crop species is as an important tool in genomics-assisted breeding aimed at optimizing flowering time and improving yield (Somerville and Koorneef, 2002; Koornneef and Meinke, 2010; Ferrier et al., 2011).

1.3 Life cycle and body plan

The life cycle of a plant consists of three main developmental phases – embryonic, vegetative, and reproductive. During embryogenesis, two apical meristems – a root apical meristem and a shoot apical meristem (SAM) are established through successive growth of a single fertilized zygote. The mature embryo is encased within a seed coat and remains dormant until germination (Capron et al., 2009; Wolpert et al., 2011). Upon germination, the root apical meristem gives rise to all below ground structures (root system) whereas the SAM produces all above ground structures (leaves, stems, branches, and flowers) in

the plants (Capron et al., 2009; Barton, 2010). Thus, the majority of development in a plant occurs post-embryonically.

Plant development of the shoot is based on the successive production of repeating units by the SAM. Each unit has three parts: a leaf, a leaf attachment site containing cells with the potential to form an axillary meristem, and a subtending stem (called an internode). In Arabidopsis, the vegetative SAM initiates leaves associated with dormant axillary meristems and internodes that do not elongate (Steeves and Sussex, 1989; Fletcher, 2002). The overall result is a basal rosette of leaves (Figure 1.1). At an appropriate time, the vegetative SAM attains reproductive competence and becomes an IM such that internodes elongate, leaf development is repressed, and axillary meristems proliferate to form lateral branches and flowers (Schultz and Haughn, 1991). Early in this transition, axillary meristems specify indeterminate lateral branches subtended by cauline leaves (Figure 1.1). When the transition is completed, axillary meristems specify determinate flowers and leaf development is repressed in full (Schultz and Haughn, 1991; Sablowski, 2007). The end product is an inflorescence shoot bearing lateral branches and flowers arranged in a regular spiral pattern on the primary stem.

1.4 The SAM is a foundation of plant aerial development

The SAM at the shoot apex houses a self-sustaining population of pluripotent stem cells. Different patterns of organ production by the SAM play a major role in generating architectural diversity in plants. Understanding how the SAM is initiated, organized, and

maintained has provided important insights into the basis of plant development (Bowman and Eshed, 2000; Barton, 2010).

1.4.1 Anatomy of the SAM

Shoot meristems are generally minute structures ensheathed within developing leaves or bracts, varying in size and shape within and between species. William (1974) reported a model for SAM organization from sections of the lupin apex and reviewed their variability in shape and dimensions. Since then, a number of models have been proposed. The majority of these models view the SAM as a compartmentalized structure consisting of two types of functional domains (Figure 1.2). The first are domains defined by varying cytoplasmic densities and cell division rates designated as the central zone (CZ), peripheral zone (PZ), and the rib zone (RZ). Superimposed on these domains is the second category consisting of clonally distinct cell layers (L1, L2, and L3) (Barton and Poethig, 1993; Bowman and Eshed, 2000; Fletcher, 2002).

The zones are synchronized to the clonal cell layers notable through arrangements of cells and patterns of cell division during organ formation. The L1 and L2, epidermal and sub-epidermal layers respectively, constitute cells of the PZ and CZ. Each of these layers is one cell thick and remains clonally distinct in derivative organs because cell division in these layers is anticlinal (i.e. perpendicular to the meristem surface) (Bernier, 1988). The inner L3 layer constitutes the RZ where cells divide in all planes (Barton, 2010). Layer L1 gives rise to the epidermis that covers all structures produced by the shoot whereas L2 and L3 layers contribute to the cortex and vascular structures. Leaves and flowers are produced

mainly from the L2 layers whereas the L3 layer contributes mainly to the stem (Fletcher, 2002; Wolpert et al., 2011). Changes in the rate and pattern of cell division are observed during the transition to flowering. For example, derepression of a developmental program by activities in the RZ are responsible for internode elongation and upward growth of the shoot in production of the inflorescence (Bernier, 1988). These observations reinforce the idea of synchronization and suggest the existence of a communication system within and between the domains and cell layers in coordination of various developmental programs.

1.4.2 Establishment of the SAM

Embryogenesis is the development of an embryo from a single fertilized zygote (Takeda and Aida, 2011; Wolpert et al., 2011). In Arabidopsis, the zygote divides asymmetrically giving rise to a cytoplasmically dense apical cell and a large vacuolated basal cell. Further divisions of apical cell give rise to the globular embryo, followed by heart, torpedo, bent cotyledon, and walking-stick stages of embryo development (Bowman and Eshed, 2000). Auxin transport to the apical cell establishes the initial apical-basal axis of the embryo upon which subsequent gene expression patterns and cell division are based (Capron et al., 2009).

A homeodomain transcription factor gene called *WUSCHEL* (*WUS)* is the earliest known marker of SAM formation whose expression is turned on in the 4 inner sub-epidermal cells of the globular embryo (Fletcher, 2002). *WUS* expression is gradually confined to the upper region of the globular embryo which gives rise to an Organizing Center required for

stem cell maintenance (Barton, 2010; Takeda and Aida, 2011). Towards the end of the globular stage, the apical portion of the embryo is patterned and stem cell identities are specified through induction of *CUP-SHAPED COTYLEDON (CUC)* and *SHOOT MERISTEMLESS* (*STM)* expression respectively. Distinct patterns of expression for these genes demarcate the embryo into central, peripheral, and rib zones (Fletcher, 2002; Barton, 2010). Analyses of *CUC* mutants have demonstrated their role in organ-organ and organ-meristem separation including their requirement for initial *STM* expression. *STM* mutants fail to maintain stem cell identity and terminate the SAM prematurely (Barton and Poethig, 1993; Long et al., 1996; Bowman and Eshed, 2000).

1.4.3 Maintenance of the SAM

Genes for SAM maintenance are activated during the heart stage of embryo development. These factors include a WUSCHEL-CLAVATA (WUS-CLV) feedback loop and THREE-AMINO-ACID-LOOP-EXTENSION (TALE) KNOTTED1-like (KNOX) and BELL1-like (BELL) homeodomain transcription factors with overlapping functions (Fletcher, 2002; Barton, 2010; Hamant and Pautot, 2010). The WUS-CLV feedback loop acts primarily to confer meristem cell fate in the CZ, whereas KNOX-BELL homeodomain proteins maintain meristem activity by inhibiting differentiation in the CZ and PZ (Clark et al., 1996; Endrizzi et al., 1996; Gallois et al., 2002; Rutjens et al., 2009; Barton, 2010).

This remarkable network maintains the SAM constant in size over the course of a life span despite lacking cell lineage constraints in determination of cell fates (Barton, 2010). For example, the SAM of a several-hundred-year-old mountain ash (*Sarbus arcuparia*) will not

differ significantly in size from the SAM of its cognate sapling nor will the obliteration of a central portion of the SAM in an Arabidopsis plant lead to death but rejuvenation of a new functional meristem from the flanks of the previous one (Steeves and Sussex, 1989; Barton, 2010). To achieve this balance, cells in the CZ remain pluripotent and divide slowly to provide daughter cells that are constantly displaced to the surrounding PZ to replenish cells lost by incorporation into lateral organs (Fletcher, 2002; Barton, 2010). Thus, positional cues in the SAM as opposed to cell lineage play a critical role in determination of cell fate.

1.4.3.1 *WUSCHEL-CLAVATA* feedback-loop

Interactions between *WUS* and *CLV* genes establish a negative feedback-loop that is required for SAM maintenance. *WUS* expression is limited to a small group of cells in the RZ known as the Organizing Center (OC) (Figure 1.2). After its synthesis in the OC, WUS homeodomain protein moves through small cytoplasmic channels called plasmodesmata to the CZ where it confers stem-cell identity and directly activates its own negative regulator *CLV3* (Yadav et al., 2011). CLV3 is a small secreted peptide belonging to a signal transduction pathway which includes the leucine rich receptor kinases CLV1 and CLV2. CLV3 is expressed in the CZ where it binds to CLV1 which is predominantly expressed in the central L3 layer of the SAM (Fletcher et al., 1999). Binding of CLV3 to CLV1 receptor activates other leucine rich receptor kinases including CLV2 initiating a signal transduction cascade that causes repression of *WUS* transcription (Schoof et al., 2000; Yadav et al., 2011). This network forms a negative feedback loop that limits *WUS* expression to the OC

in maintaining pluripotency and size of the SAM. A mutation in *WUS* leads to depletion of the stem cell population and premature termination of the SAM (Fletcher, 2002; Barton, 2010). Conversely, a mutation in any of the *CLV* genes leads to enlargement of the SAM and supernumerary organs (Brand et al., 2000; Fletcher, 2002). The communication between *WUS* expressing cells in the OC and the overlying stem cell population demarcated by the *CLV3* expression domain establishes one of the most fundamental structures in plant development, a self-maintaining stem cell population with organogenesis potential to last a life span.

1.4.3.2 A TALE of SAM maintenance

Another layer in SAM maintenance is imposed by members of the TALE superfamily of homeodomain proteins. TALE members are key developmental regulators in plants with roles in meristem maintenance, cell differentiation, and boundary maintenance. The TALE superfamily comprises KNOX and BELL subfamilies (Hamant and Pautot, 2010). STM is the founding member of the KNOX family in Arabidopsis and plays an essential role in SAM formation during embryogenesis and SAM maintenance during post-embryonic development (Long et al., 1996; Hamant and Pautot, 2010). Strong *stm* mutants are unable to replenish their stem-cell population and terminate after the initiation of 1-2 leaves (Long et al., 1996). Double mutant analyses show that two closely related KNOX members expressed in the meristem and at boundaries flanking the meristem respectively, BREVIPEDICELLUS (BP) and KNAT6, contribute redundantly with STM in

meristem maintenance since their inactivation enhances the meristem defects of weak *stm* mutants (Bryne et al., 2002; Belles-Boix et al., 2006).

KNOX and BELL members function as heterodimers which is important for binding site selection, nuclear localization, and protein stability (Bellaoui et al., 2001; Rutjens et al., 2009). At least three BELL members (PNY, PNF, and ATH1) are expressed in the SAM where they interact with STM and function in SAM maintenance (Rutjens et al., 2009).

1.4.4 Lateral organ boundaries

Lateral organ boundaries are specialized domains with restricted growth that separate the SAM from differentiating lateral organs on its flanks (Aida and Tasaka, 2006a, 2006b; Rast and Simon, 2008). Boundaries contain cells with a slower rate of division than surrounding cells resulting in the formation of a groove that separates organs from the meristem and from adjacent initiating organs (Rast and Simon, 2008). As development proceeds, the boundary is a source of axillary meristems that give rise to shoots including lateral branches and flowers (Rast and Simon, 2008). Separation events including abscission and seed or pollen dehiscence also take place at boundaries (Aida and Tasaka, 2006a, 2006b; Rast and Simon, 2008). Over the years a number of genes that participate in boundary formation, maintenance, and differentiation have been identified in Arabidopsis.

1.4.4.1 NAC domain-containing transcription factors

NAC domain containing transcription factors confer boundary identity in all known species of plants. In Arabidopsis, this activity is provided by CUC1, CUC2, and CUC3 whose

members are also responsible for activation of *STM* during SAM formation in the embryo (Aida et al., 1997; Vroemen et al., 2003; Aida and Tasaka, 2006a, 2006b). Activation of STM establishes a negative feedback loop restricting *CUC* expression to the periphery in establishing a boundary that separates the meristem and embryonic leaves (Aida et al., 1999). Progressive loss of CUC activity in Arabidopsis causes the fusion of cotyledons and prevents establishment of the embryonic SAM showing a dual function in organ separation and meristem maintenance (Aida and Tasaka, 2006a, 2006b; Takeda and Aida, 2011).

1.4.4.2 *BLADE-ON-PETIOLE* genes

BTB-ankyrin transcription factors discovered in Arabidopsis are also required in maintaining and specializing lateral organ boundaries in plants (Ha et al., 2004; Hepworth et al., 2005; Norberg et al., 2005; Khan et al., 2014). Genetic studies suggest that *BOP1/2* function downstream and/or in parallel with *CUC* genes in performing this function. Loss of function *bop1 bop2* mutations do not alter *CUC* expression but exhibit organ fusions during post-embryonic development resulting from ectopic growth at the boundary (Khan et al., 2014). BOP activity is also essential for the initiation and differentiation of axillary meristems and for the differentiation of abscission zones (Hepworth et al., 2005; Norberg et al., 2005; McKim et al., 2008; Xu et al., 2010; Khan et al., 2014).

Leafy petioles are a hallmark of the *bop1 bop2* mutant phenotype. This phenotype is due to ectopic meristem activity resulting from reactivation of KNOX genes *BP* and *KNAT2* in leaves (Ha et al., 2003; Ha et al., 2007; Ha et al., 2010). BOP1/2 indirectly repress KNOX

gene expression in leaves through activation of *ASYMMETRIC LEAVES2* whose product functions in a repression complex (Guo et al., 2008; Jun et al., 2010; Lodha et al., 2013).

BOP1/2 are known to activate several other boundary genes including *KNAT6* and *ATH1* whose interacting products form a KNOX-BELL heterodimer (Rutjens et al., 2009; Li et al., 2012). Ectopic expression of these genes restricts growth and antagonizes meristem activity during inflorescence development generating late flowering plants with shortened internodes, clustered fruits, and woody stems (McKim et al., 2008; Shi et al., 2011; Khan et al., 2012a; Khan et al., 2012b; Khan et al., 2014). This module also functions in fruit patterning and abscission (Ragni et al., 2008; Khan et al., 2012b; Shi et al., 2011).

1.4.4.3 A TALE of boundaries

Genetic studies show that KNOX-BELL family members in the meristem position organ boundaries *via* repression of lateral organ boundary genes. This is first seen in embryos where STM restricts *CUC2* expression to the axil of cotyledons in conjunction with auxin-based signals from the leaf (Takeda and Aida, 2011). This is also seen in *stm* mutants, where *BOP1/2* expression expands to the meristem region correlating with SAM arrest after the production of 1-2 leaves (Jun et al., 2010). At least three BELL factors with overlapping functions (PNY, PNF, and ATH1) are involved in SAM maintenance with STM based on genetic studies. In *pny pnf* double mutants, about 50% of seedlings terminate after the initiation of 5-6 leaves (Rutjens et al., 2009; Ung et al., 2011). Meristem activity is restored by inactivation of BOP1/2 or KNAT6 consistent with *BOP1/2* and *KNAT6* misexpression in *pny pnf* apices (Tabb, 2012). *KNAT6* expression is strongly localized to

boundaries at all stages of development (Belles-Boix et al., 2006; Ragni et al., 2008; Khan et al., 2012b). In contrast, *ATH1* is down-regulated in the SAM at the transition to flowering and expressed in boundaries at the base of floral shoots and floral organs during reproductive development contributing to inflorescence architecture and abscission (Proveniers et al., 2007; Gómez-Mena and Sablowski, 2008; Rutjens et al., 2009; Khan et al., 2012a). Loss-of-function alleles of *ath1* also rescue *pny pnf* defects in flowering suggesting that it is part of the pathway with BOP1/2 and KNAT6 (Tabb, 2012; Khan et al., 2015). The differential function of KNOX and BELL factors at different stages of development suggests that their activities are highly dependent on context.

1.5 Reproductive Development

1.5.1 Overview

The switch from vegetative growth to flowering is a tightly controlled developmental decision in plants. Flowering-time is based on meristem competence to respond to interacting signals from endogenous and environmental pathways that converge at the SAM to promote acquisition of IM fate (Amasino and Michaels, 2010; Srikanth and Schmid, 2011; Andrés and Coupland, 2012). This process known as floral evocation results in new patterns of growth at the shoot apex and the production of an inflorescence (Bernier, 1988; Smith et al., 2004).

Floral repressors in the SAM block meristem competence to flower during vegetative stages of development (Bernier, 1988; Yant et al., 2009). Major pathways for promotion of flowering work in two ways: *via* down-regulation of floral repressors in the meristem

and *via* promotion of expression of genes that promote IM and floral meristem (FM) identity (Figure 1.3).

Commonly studied environmental cues for flowering include temperature and photoperiod. Endogenous pathways function independently of environmental signals and are called “autonomous”. Five major pathways for promotion of flowering have been identified. These include a vernalization pathway that promotes flowering after exposure to prolonged cold, an autonomous pathway, a miR156-SPL-miR172 module that integrates age-related, sugar, and stress signals, a photoperiod pathway that promotes flowering in response to long-day photoperiods, and gibberellin (GA) for promotion of flowering when other inductive signals are absent (Turck et al., 2008; Srikanth and Schmid, 2011; Wang, 2014). Inputs from these various pathways converge to control the expression of a small number of genes with floral “integrator” activity which directly promote the expression of genes that confer IM and FM identity (Parcy, 2005; Amasino and Michaels, 2010; Andrés and Coupland, 2012).

The flowering behaviour in Arabidopsis comprises winter annual and summer annual subtypes. Winter annual ecotypes are late-flowering unless exposed to a winterizing period called vernalization that suppresses transcript abundance of the floral repressor FLOWERING LOCUS C (FLC). FLC is also down-regulated by the autonomous pathway. Long-day (LD) photoperiods accelerate flowering by triggering the stabilization and accumulation of CONSTANS (CO) protein in leaves, which in turn directs the transcription of *FLOWERING LOCUS T (FT)*. FT is a small phosphatidylethanolamine-binding protein,

which is synthesized in leaves and transported through phloem to the SAM where it promotes flowering (Kardailsky et al., 1999; Kobayashi et al., 1999; Corbesier et al., 2007; Jaeger and Wigge, 2007; Mathieu et al., 2007). FT interacts with the bZIP transcription factor FD, which is expressed in the SAM to activate genes conferring inflorescence meristem identity, including *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS1/AGAMOUS-LIKE20 (SOC1/AGL20)*, *AGL24*, and *FRUITFULL* (*FUL*) (Abe et al., 2005; Teper-Bamnolker and Samach, 2005; Wigge et al., 2005). These factors together with FT directly promote the expression of floral meristem identity genes *LEAFY* (*LFY*), *APETALA1* (*AP1*), and *CAULIFLOWER* (*CAL*) whose products acting on axillary meristems confer floral fate (Bowman et al., 1993). GA is critical for flowering under short days by directly activating floral integrator genes at the shoot apex (Mutasa-Göttgens and Hedden, 2009).

Finally, down-regulation of microRNA156 (miR156) with age stabilizes mRNA encoding transcription factors SQUAMOSA PROMOTER BINDING PROTEIN-LIKE (SPL3), SPL4, and SPL5 which function in conjunction with FT-FD to initiate flowers via direct activation of *AP1, LFY,* and *FUL* (Yamaguchi et al., 2009; Jung et al., 2012; Wang, 2014). The components of these different pathways are discussed in detail in the following sections.

1.5.2 Reproductive competence

Reproductive competence depends on the ability of the SAM to perceive and respond to signals for flowering (Bernier, 1988). Early in the vegetative phase, the SAM lacks competence to flower. It initiates leaves with juvenile characteristics denoted by lack of

trichomes on the abaxial surface. As development progresses, broader adult leaves are formed, denoted by trichomes on both surfaces. These morphological changes reflect a progression from juvenile to adult phases of vegetative development. These changes are paralleled by changes in reproductive competence at the SAM (Telfer et al., 1997; Bergonzi and Albani, 2011). Only the adult vegetative meristem is competent to perceive and respond to floral inductive signals.

The transition from juvenile to adult phases of vegetative development is tightly controlled to ensure that flowering occurs only when the plant has accumulated sufficient internal resources to support reproduction (Poethig, 1990). Genetic studies have defined a handful of genes and microRNAs (miRNAs) that regulate vegetative phase-change including *EMBRYONIC FLOWER (EMF)*, *TERMINAL FLOWER (TFL1),* and miRNA156 (Pose et al., 2012; Wang, 2014). *EMF* mutants initiate a flower straight after germination whilst *TFL1* mutants produce a terminal flower after a short-lived vegetative phase. EMF exerts its function by preventing premature expression of flowering genes based on chromatin structure. TFL1 exerts its function by competitive binding to the bZIP factor FD required by FT for recruitment to DNA (Liljegren et al., 1999; Ratcliffe et al., 1999; Pose et al., 2012). TFL1 further represses *LFY* and *AP1* expression in the IM so that the meristem remains indeterminate and capable of producing numerous floral meristems (Liljegren et al., 1999; Ratcliffe et al., 1999; Jaeger et al., 2013).

1.5.3 The miRNA156-SPL-miR172 module

Superimposed on this network is a timing mechanism composed of opposing gradients of microRNAs. A temporal gradient of miRNA156 inhibits reproductive competence by preventing transcript accumulation of SPL factors required in lowering the abundance of AP2-like floral repressors *via* transcriptional activation of miR172 (Wu et al., 2006; Wang, 2009; Yamaguchi et al., 2009; Yamaguchi and Abe, 2012; Wang, 2014). Age, stress, and sugar are three factors that directly control the abundance of miR156 to regulate flowering-time (Wang, 2009; Proveniers, 2013; Cui et al., 2014; Stief et al., 2014; Wang, 2014)

The miR156-SPL-miR172 module is an ancient and conserved pathway in plants (Wu and Poethig, 2006; Axtell and Bowman, 2009; Wang, 2009; Yamaguchi et al., 2009; Huijser and Schmid, 2011; Wang, 2014). Pioneering studies in Arabidopsis observed that the temporal expression pattern of miRNA156 accumulation coincides with juvenile-to-adult phase transition which in turn controls reproductive competence at the SAM (Wu and Poethig, 2006; Yamaguchi and Abe, 2012). These studies showed that up-regulation of miR156 transcript prolongs the vegetative phase leading to a delay in flowering, whereas up-regulation of miRNA172 transcript accelerates progression through the vegetative phase resulting in early flowering (Bergonzi and Albani, 2011). Thus, miRNA156 acts to repress reproductive competence whereas miRNA172 acts to promote it.

Studies show that miR156 levels decline with age leading to a concomitant increase in abundance of *SPL* transcripts in leaves and the shoot apex (Wu and Poethig, 2006; Wang, 2009; Wu et al., 2009). The Arabidopsis genome encodes 11 miR156-regulated *SPL* genes

comprising subgroups represented by SPL3 (SPL3/4/5) and SPL9 (SPL2/6/9/10/11/13/13-like and 15) (Wang, 2014). SPLs promote flowering by direct positive transcriptional regulation of miR172. miRNA172 down-regulates the abundance of a family of AP2-like floral repressors that counteract SPL3 induction of floral meristem identity genes *LFY* and *AP1* and SPL9 induction of *FT*, *SOC1* and *FUL* (Wu et al., 2009; Yamaguchi et al., 2009; Zhu and Helliwell, 2011; Matsoukas et al., 2012; Wang, 2014). Consistent with these observations, transgenic lines expressing SPLs with mutations in the miRNA156 target site have elevated levels of miRNA172 and flower extremely early with ectopic vegetative adult traits (Wu and Poethig, 2006; Wang, 2009; Bergonzi and Albani, 2011).

Recent data show that stress signals are also integrated through this module. Cui et al. (2014) showed that delayed flowering in response to stress stimuli correlates with increased abundance of miR156 leading to down-regulation of *SPL9*. Compatible with this, plants overexpressing miR156 show increased tolerance to stress and are very late flowering. Along similar lines, Stief et al. (2014) showed that induction of miR156a-f isoforms by heat stress correlates with down-regulation of *SPL9*-like transcripts (*SPL2*, *SPL9*, *SPL11*) and delayed flowering. Finally, it is shown that miR156-SPL3 play a role in delaying flowering under cool ambient temperatures via regulation of *FT* (Kim et al., 2012).

The miR156-SPL-miR172 module is also a system for the integration of nutritional signals. A developmental decline in miR156 leading to *SPL* accumulation is partially mediated by sugars produced by photosynthesis (Proveniers, 2013; Yang et al., 2013; Yu et al., 2013).

Reproductive development requires sufficient mobile carbohydrate to fuel an increased rate of cell division in aerial tissues for production of inflorescences, flowers, and seeds. Plants measure sugar status in leaves and in the shoot apex by monitoring the abundance of trehalose-6-phosphate (T6P), a trace metabolite synthesized by trehalose-6-phoshate synthase1 (TPS1) from glucose-6-phosphate and uridine diphosphate (UDP)-glucose (Matsoukas et al., 2012; Wahl et al., 2013). *TPS1* is upregulated in shoot apices during the transition to flowering leading to production of T6P which in turn promotes *SPL3/4/5* expression in the SAM *via* miR156-dependent and –independent pathways (Wingler et al., 2012; Wahl et al., 2013).

1.5.4 Pathways targeting FLOWERING LOCUS C

The MADS-box transcription factor FLOWERING LOCUS C (FLC) is another key repressor of meristem competence to flower. FLC abundance is directly controlled by vernalization and the autonomous pathway which down-regulate expression to promote flowering. Constitutive expression of *FLC* can delay or completely block flowering in a dosage-dependent manner (Michaels and Amasino, 1999; Sheldon et al., 1999). FLC is the founding member of small clade of MADS-box floral repressors that includes SHORT VEGETATIVE PHASE (SVP) (Hartmann et al., 2000; Li et al., 2008). FLC and SVP form a complex that directly repress the expression of key floral integrator genes including *SOC1*, *FT*, and *FD* (Hepworth et al., 2002; Helliwell et al., 2006; Searle et al., 2006). In winter annual ecotypes of Arabidopsis with a functional allele of *FLC*, plants must be exposed to prolonged cold (vernalization) to promote and maintain silencing of *FLC* in order to flower.

Laboratory strains of Arabidopsis including Col-0 used in my study are summer annuals with weak alleles of *FLC* that permit flowering without a requirement for vernalization (Michaels and Amasino, 1999; Shindo et al., 2005; Werner et al., 2005). In these ecotypes, *FLC* expression in the meristem is down-regulated by members of the autonomous pathway (Simpson, 2004). Mutations in genes of the autonomous pathway cause delayed flowering regardless of photoperiod due to elevated levels of FLC in the meristem. Following down-regulation of *FLC*, the meristem is competent to respond to floral inductive signals generated by long day photoperiods and gibberellin (Henderson and Dean, 2004; He and Amasino, 2005).

1.5.5 Photoperiod pathway

Long-day photoperiods are a powerful stimulus for flowering in Arabidopsis via light-dependent stabilization of the zinc-finger transcription factor CONSTANS (CO). Accumulation of CO at the end of a long day photoperiod directly activates the expression of *FT* in leaves and *SOC1* at the shoot apex (Samach et al., 2000). FT is synthesized in phloem companion cells of the leaf and loaded into the phloem for transport to the shoot apex where it binds to FD for direct activation of *AP1* (Abe et al., 2005; Wigge et al., 2005; Corbesier et al., 2007). In the SAM, SOC1 directly activates *LFY* (Liu et al., 2008). Expression of *LFY* and *AP1* in axillary meristems confers floral fate, a commitment step in the conversion of shoot to flowers (Wagner et al., 1999; Abe et al., 2005; Wigge et al., 2005).

1.5.6 Gibberellins

GA is a leaf-produced plant hormone whose activity is critical for flowering and internode elongation in non-inductive photoperiods (Mutasa-Göttgens and Hedden, 2009). At the transition to flowering, GA content increases 100-fold at the shoot apex leading to degradation of DELLA repressors of GA signalling (Eriksson et al., 2006; Harberd et al., 2009; Schwechheimer and Willige, 2009). Under short days, GA is critical for promotion of *SOC1* and *LFY* expression at the shoot apex. Under long days, the photoperiod pathway fulfills this function (Mutasa-Göttgens and Hedden, 2009). GA additionally acts through the miR156-SPL-miR172 module to promote flowering (Porri et al., 2012; Galvão et al., 2012; Yu et al., 2012). GA/DELLA are shown to regulate *SPL3/4/6/9* transcription at the shoot apex independent of SOC1 (Galvão et al., 2012; Porri et al., 2012). Physical interaction of DELLAs with SPL9 factors provides an additional layer of post-transcriptional control. RGA DELLA binding to SPL9 interferes with activation of MADS-box flowering genes at the shoot apex and activation of miR172b in leaves which maintains AP2 and AP2-like repression of stem elongation and flowering (Yu et al., 2012; Wang, 2014). Thus, GA is a factor in integrating autonomous and age-related signals for flowering.

1.5.7 SAM-dependent integration of flowering signals

Inputs from flowering-time pathways converge at the SAM to up-regulate the expression of a network of genes with floral integrator activity including *LFY*, *FT*, and *SOC1* (Parcy, 2005). Targets include MADS-box flowering-time genes *SOC1*, *FUL*, and *AGL24* which encode key determinants of IM and FM identity genes (Michaels et al., 2003; Yu et al., 2004; Teper-Bamnolker and Samach, 2005; Liu et al., 2007).

Members of the TALE class of homeodomain transcription factors which promote stem cell fate are essential for SAM responsiveness to floral inductive signals (Hamant and Pautot, 2010; Hay and Tsiantis, 2010). Meristems lacking PNY and PNF activities support the production of leaves but are unable to support the production of internodes or flowers (Smith et al., 2004).

The *pny pnf* meristem changes shape in response to floral inductive signals and inflorescence meristem identity genes *SOC1* and *FUL* are up-regulated but *FT-FD* levels are reduced and floral meristem identity genes *LFY*, *AP1,* and *CAL* are not expressed (Smith et al., 2004; Kanrar et al., 2008). The basis of this phenotype is only partly understood. Ectopic expression of *LFY* in *pny pnf* mutants partially rescues flowering at axillary meristems whilst ectopic expression of *FT* fails to rescue flowering and only partially restores internode elongation at great length suggesting that FT requires PNY-PNF to initiate flower development (Kanrar et al., 2008). Additional data show that STM functions in association with PNY-PNF to specify flowers *via* promotion of *LFY* expression (Kanrar et al., 2006; Kanrar et al., 2008). This has led to the proposal that STM/PNY-PNF function together with flowering-time products FT-FD and AGL24-SOC1 to initiate development of reproductive structures, flowers and internodes (Smith et al., 2011). More recently, PNY-PNF were shown to promote the expression of SPL3, 4, and 5 transcription factors that direct activation of floral-meristem identity genes in parallel with FT-FD (Lal et al., 2011). Compatible with this, miR156 is up-regulated in *pny pnf* apices. Ectopic expression of *SPL4* in *pny pnf* restores accumulation of *LFY* and *AP1*

transcripts and partially restores flower formation (Lal et al., 2011). However, none of these mechanisms identified to date fully explain the basis of *pny pnf* meristem defects.

1.6 Thesis rationale

Our lab has investigated the role of PNY-PNF in flowering and shown that defects in meristem maintenance and IM activity are caused by misexpression of a set of lateral organ boundary factors comprising BOP1/2 and a pair of downstream effectors, the KNOX-BELL homeodomain factors KNAT6 and ATH1. Inactivation of genes in this module fully rescues *pny pnf* defects in meristem maintenance, internode elongation, and flowering. Understanding how this module blocks flowering was a key focus of my thesis studies.

A steroid induction system was used to show that BOP1 directly activates ATH1 expression. However, binding sites for BOP1 on the *ATH1* promoter were unknown. BOP1 and BOP2 belong to a small transcription factor family in Arabidopsis whose founding member is the defense regulator NON-EXPRESSOR of PATHOGENESIS-RELATED GENES1 (NPR1). Members of this family contain two conserved protein-protein interactions motifs: an N-terminal BTB/POZ (for Broad-Complex, Tramtrack, and Bric-a-Brac/POX virus and Zinc finger) domain for dimerization and a C-terminal set of four ankryin repeats that mediate interactions with TGA bZIP family members for recruitment to DNA (Després et al., 2000; Després et al., 2003; Hepworth et al., 2005; Xu et al., 2010; Khan et al., 2014). Five clades of TGA bZIP factors encode ten family members in the Arabidopsis genome (Jakoby et al., 2002; Gatz, 2013). Unpublished data show that clade I factors (TGA1 and

TGA4) are expressed at lateral organ boundaries and are required by BOP2 to exert changes in inflorescence architecture (Khan, 2013; Devi, 2014). Therefore, clade I factors are potential candidates for recruitment of BOP1 to the *ATH1* promoter in regulation of flowering.

To gain further insight into how this module blocks flowering, microarray was used to analyze the transcriptome of *BOP1* overexpressing plants. Analyses of these data led to the hypothesis that stress signals communicated by jasmonic acid (JA) antagonize gibberellin either directly or indirectly *via* the miR156-SPL-miR172 module to delay flowering. JA antagonism of growth in the literature is variously linked to inhibition of GA biosynthesis, catabolism, and/or signaling (Magome et al., 2004; Magome et al., 2008; Heinrich et al., 2012; Yang et al., 2012) in addition to regulation of SPL transcription factor activity in the miR156-SPL-miR172 module (Wang, 2014).

1.7 Summary and Model

In summary, *PNY* and *PNF* in the IM repress lateral organ boundary genes including BOP1/2 and ATH1/KNAT6 to regulate SAM maintenance and competence to flower. We hypothesize that BOP1/2 bind to the *ATH1* promoter via clade I TGA factors TGA1 and TGA4 which have an expression pattern at lateral organ boundaries. We further hypothesize that genes in this unit block reproductive development in part by activating stress pathways that promote JA biosynthesis which directly or indirectly interferes with GA signals and/or the miR156-SPL-miR172 module to block IM activity.

My thesis tested the following hypotheses

1. BOP directly activates *ATH1* by binding to bZIP sites on its promoter
2. Clade I bZIP factors are part of the BOP module that block flowering
3. The module blocks flowering in *pny pnf* mutants in part through positive regulation of JA accumulation which counteracts GA promotion of flowering.

Figure 1.1 Architecture of the model plant *Arabidopsis thaliana*

(A) **Vegetative phase:** The SAM initiates leaves in a spiral pattern without internode elongation. A compact rosette of leaves is formed at the base of the plant. Axillary meristems are dormant but outgrow during the reproductive phase to form a paraclade. (B) **Reproductive phase:** Conversion of the SAM to an IM results in the production of an inflorescence. Leaf development is repressed. Internodes elongate and axillary meristems are activated. In the first few modules produced by the IM, axillary meristems in the axils of cauline leaves specify secondary inflorescences. When the transition is complete, leaf development is fully repressed and axillary meristems are converted to floral meristems that form single flowers that are fertilized and mature into an elongated fruit (silique). Scale bar, 1.5 cm.

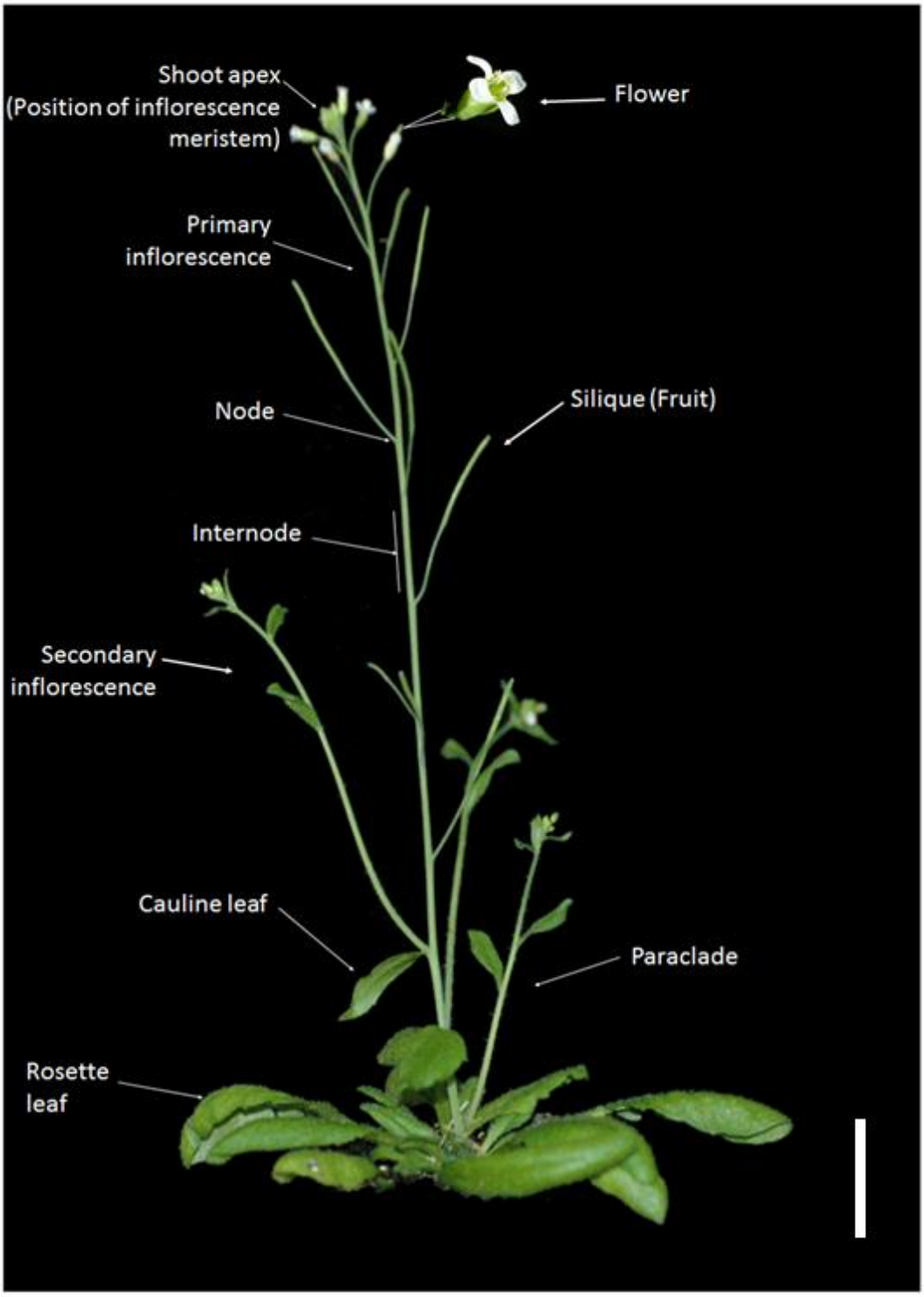

Shoot apex
(Position of inflorescence meristem)
Flower
Primary inflorescence
Node
Silique (Fruit)
Internode
Secondary inflorescence
Cauline leaf
Paraclade
Rosette leaf

Figure 1.2 Organization of the SAM in *Arabidopsis thaliana*

The SAM is a compartmentalized structure consisting of three functional domains (CZ, PZ, and RZ) superimposed on three clonal layers (L1, L2, and L3).

(A) **Domains**: The central zone (CZ) in RED is centrally located at the shoot apex and contains a reservoir of stem cells. The CZ is flanked by the peripheral zone (PZ) in pale PINK which is responsible for incorporation of stem cell derivatives into lateral organs (leaves and flowers). The rib zone (RZ) in WHITE is located beneath the CZ where it participates in formation of internal stem tissues. The Organizing Center (OC) in BLUE consists of *WUS* expressing cells that confer stem cell identity upon overlying cells in the CZ. The lateral organ boundaries in GREEN are located between the SAM and organ primordia in YELLOW and contain slowly dividing cells that maintain growth separation between undifferentiated cells in the meristem and newly forming lateral plant organs.

(B) **Clonal layers**: The epidermal layer (L1) and sub-epidermal layer 2 (L2) constitute cells in the PZ and CZ. Each of these layers is one cell thick. Cell division in these layers is anticlinal (parallel to the meristem surface) thereby maintaining the ordered layer structure of their derivative lateral organs. The underlying layer 3 (L3) divides in all planes resulting in unordered assemblies of cells that form the bulk of stem tissues.

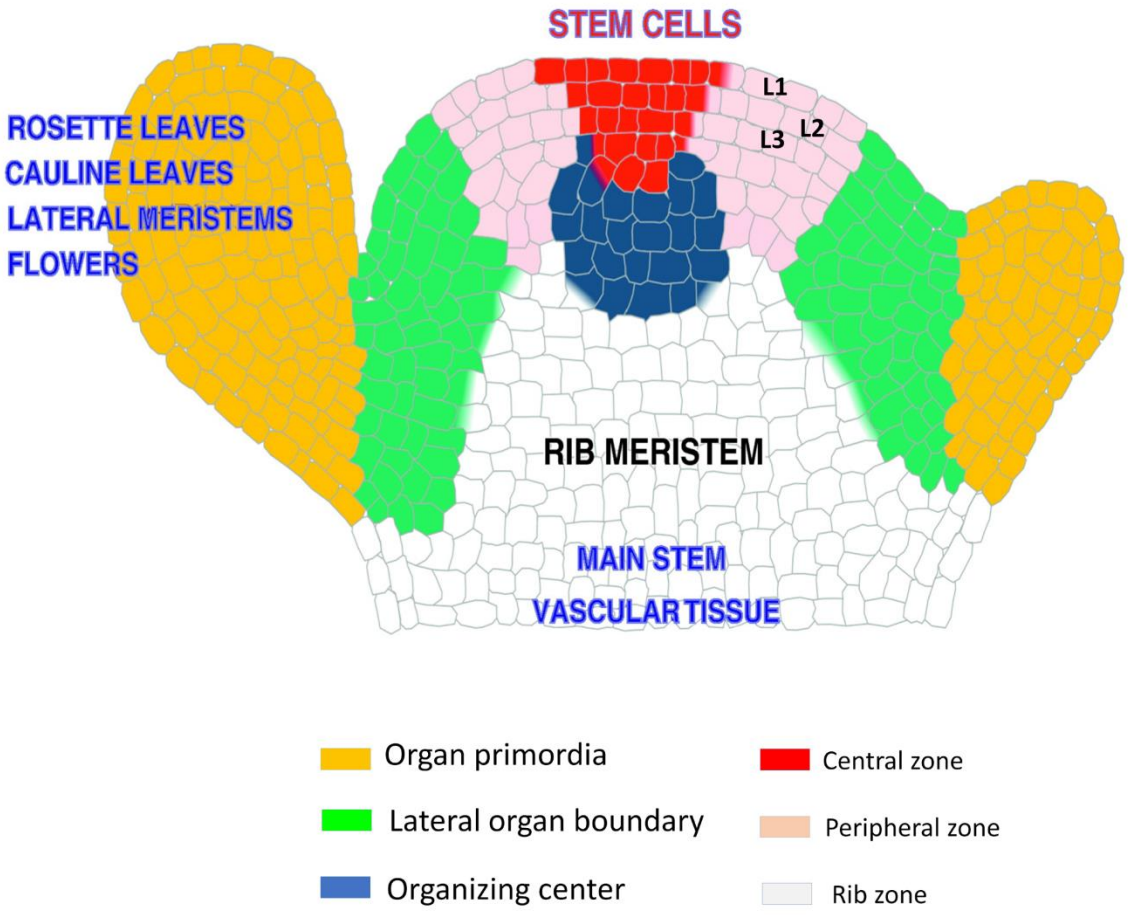
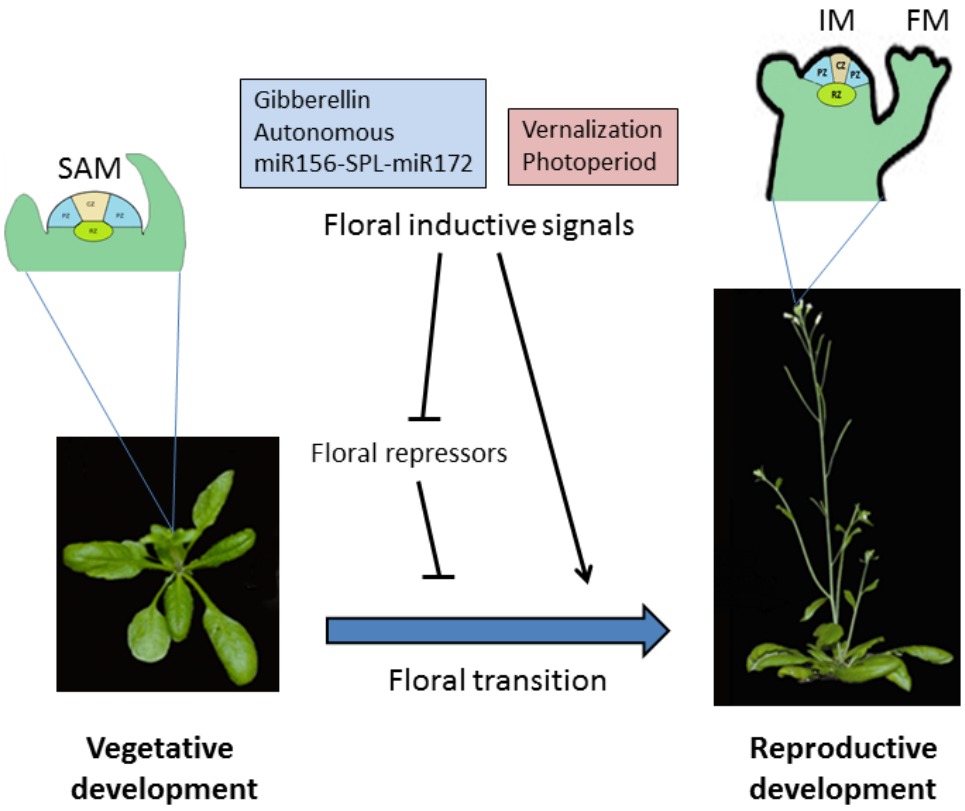

STEM CELLS
L1
L2
L3
ROSETTE LEAVES
CAULINE LEAVES
LATERAL MERISTEMS
FLOWERS
RIB MERISTEM
MAIN STEM
VASCULAR TISSUE
Organ primordia
Lateral organ boundary
Organizing center
Central zone
Peripheral zone
Rib zone

Figure 1.3 Simplified scheme for control of flowering in *Arabidopsis thaliana*

Floral repressors at the shoot apex block reproductive competence during vegetative development. Floral inductive signals from a combination of endogenous (pink) and exogenous (blue) pathways converge at the SAM to regulate the abundance of a small number of genes with floral integrator activity. Floral integrators lower the abundance of floral repressors to promote meristem competence to flower. In parallel, floral integrators promote IM identity and the conversion of axillary meristems to floral shoots. These activities are co-ordinated with proliferation of cells in the rib zone of the meristem to make internodes.


IM
FM
SAM
Gibberellin
Autonomous
miR156-SPL-miR172
Vernalization
Photoperiod
Floral inductive signals
Floral repressors
Floral transition
Vegetative development
Reproductive development

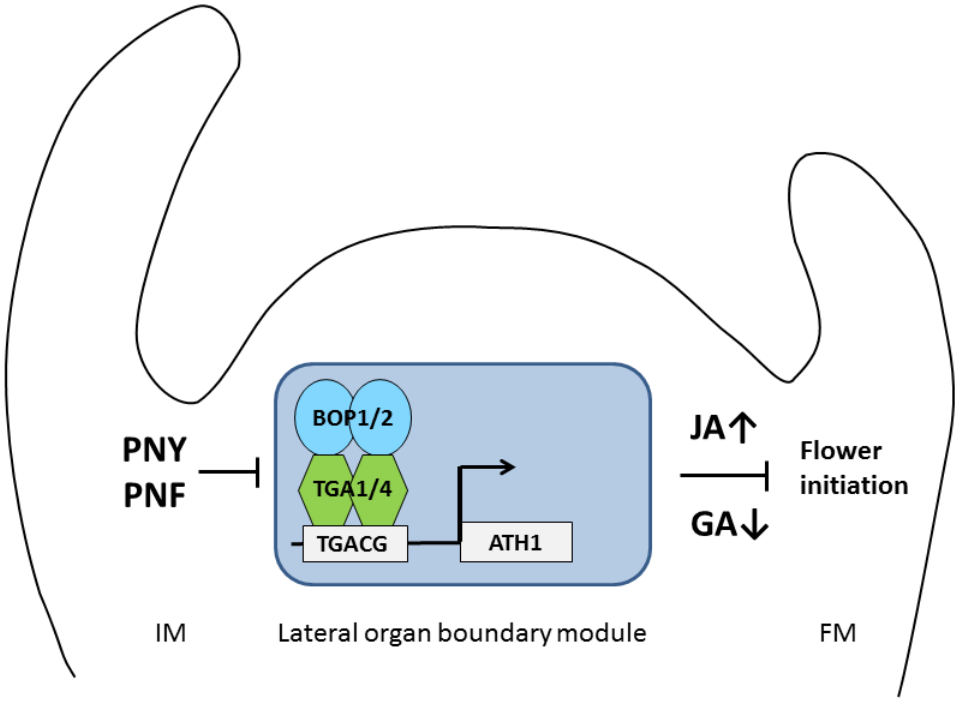


Figure 1.4 Hypothesis and model

PNY and PNF in the IM confine expression of *BOP1/2* to the lateral organ boundaries in promoting SAM maintenance and competence to flower. We hypothesize that BOP1/2 bind to the *ATH1* promoter via TGACG-containing bZIP-binding elements occupied by clade I TGA factors. We further hypothesize that genes in this unit activate stress pathways that promote JA biosynthesis which directly or indirectly interferes with GA signals to block flower initiation *via* the miR156-SPL-miR172 module (not shown).

CHAPTER 2

MATERIALS AND METHODS

2.1 Plant materials and growth conditions

Wild-type was the Columbia-0 (Col-0) ecotype of *Arabidopsis thaliana*. Plants were grown on soil or *in vitro* in growth chambers at 21°C under continuous light (24h light, intensity 100 µmol m^{-2} s^{-1}), long day (LD; 16h light), or short day (SD; 8h light) conditions. Mutant lines were obtained from the Arabidopsis Biological Resource Center (https://abrc.osu.edu/) or Nottingham Arabidopsis Stock Centre (http://nasc.nott.ac.uk). The *pny-40126* (SALK_40126), *pnf-96116* (SALK_96116), *bop1-3* (SALK_012994), *bop2-1* SALK_075879), *ath1-1* (GABI-KAT_114A12), *ath1-3* (SALK_113353), *tga1-1* (SALK_028212) and *tga4-1* (SALK_127923), and *aos* mutants have been described previously (Park et al., 2002; Smith and Hake, 2003; Smith et al., 2004; Hepworth et al., 2005; Proveniers et al., 2007; Kesarwani et al., 2007; Gómez-Mena and Sablowski, 2008). The *ath1-4* mutant was a gift from Lin Xu (Li et al., 2012). The activation-tagged *bop1-6D* overexpression line has been described previously (Norberg et al., 2005). The *35S:KNAT6* overexpression line was also described previously (Shi et al., 2011). Double and triple mutant combinations were obtained by crossing. PCR genotyping was used to confirm all mutant combinations.

Seeds were surface sterilized using 70% ethanol and a solution of 50% (v/v) household bleach (5% sodium hypochlorite) and 0.5% (w/v) sodium dodecyl sulphate (SDS). Seeds were sown on agar plates containing AT minimal media (Haughn and Somerville, 1986) or on steam-sterilized soil (Promix BX Black, Premier Horticulture, Rivière-du-Loup, QC) fertilized with a solution of 1 gram per litre of 20-20-20 plant fertilizer (Plant Prod, Brampton, ON). Seeds were incubated at 4^0C for 2 days in the dark (tin foil wrapped) to break dormancy and then placed in light to promote germination. Seedlings on agar plates

were transplanted to soil in 72-well propagation trays or in 3.5 inch square pots as appropriate and watered daily.

2.2 Genotyping

Genomic DNA was extracted from leaves as described (Edwards et al., 1991). The strategy for genotyping T-DNA insertion mutants was as described (www.signal.salk.edu). Genotyping tests for *pny-40126* (Smith and Hake, 2003), *pnf-96116* (Smith et al., 2004), *ath1-1* (Proveniers et al., 2007), *ath1-3* (Gómez-Mena and Sablowski, 2008), *tga1-1* and *tga4-1* (Devi, 2014), were as previously described. The *aos* mutant (Park et al., 2002) was genotyped using primers AOS-F1 and TJRB to detect the T-DNA insertion and primers AOS-F1 and CYP74A1-R1 to detect the wild-type genomic locus. For genotyping *ath1-4*, primers ath1-4dCAPS-F and ath1-4dCAPS-R were used to amplify a 198-bp product from genomic DNA. The *ath1-4* PCR product is cleaved by *Ssp*I to yield a 173-bp fragment. Genotyping primers were as listed in Table 2.1

2.3 Construction of *D35S:ATH1* and *ATH1p:GUS* transgenic lines

To create the *D35S:ATH1* transgene, the coding region of *ATH1* was amplified by polymerase chain reaction (PCR) using gene-specific primers ATH1-CDS-F1 and ATH1-CDS-R1 using cloned cDNA as the template. For all cloning steps involving PCR amplification, iProof was used as the polymerase (BioRad). The resulting product was modified to contain dATP overhangs and transferred to the Gateway-compatible entry vector pCR8/GW/TOPO (Invitrogen). Cloned inserts were sequenced to ensure fidelity. LR clonase (Invitrogen) was used to move the insert to a pSM-3 based destination vector

(Carl Douglas lab, unpublished) containing a double 35S CaMV promoter (D35S) and a Nos terminator. Wild-type plants were transformed by floral dipping (Clough and Bent, 1998) using Agrobacterium strain C58C1 pGV101 pMP90 (Koncz and Schell, 1986). Hygromycin-resistant primary transformants were selected on agar plates. Phenotypes were scored in the T1 generation.

To create *ATH1* promoter fusions to a GUS reporter gene (*ATH1p:GUS*), 1.0-kb, 2.0-kb, 3.0-kb, and 3.3-kb fragments including the *ATH1* translation start site were amplified by PCR from genomic DNA template (BAC MSD21). The resulting fragments were used to make in-frame fusions to the coding region of the *uidA* gene (GUS). Primers incorporating *Bam*HI and *Nco*I restriction sites at their 5' ends facilitated directional cloning. Products were cloned into pCR-BluntII-TOPO (Invitrogen) for propagation. Inserts were released by digestion with *Bam*HI and *Nco*I and ligated into the corresponding sites of pGCO:GUS (Hepworth et al., 2002). Agrobacterium was co-transformed with pSOUP (Hellens et al., 2000). Col-0 plants homozygous for a *D35S:BOP1-GR* transgene (Khan, 2013) were transformed by floral dipping. Glufosinate-ammonium resistant primary transformants were selected on soil and monitored for GUS activity in the T1 generation. Only the 2.0-kb and 3.3-kb promoter fusions showed GUS activity and were further analyzed. Cloning primers were as listed in Table 2.1.

Three representative lines for the 2.0-kb and 3.3-kb ATH1p:GUS in *D35S:BOP1-GR* lines were chosen for further analysis. Final experiments were conducted using homozygous lines.

2.4 Beta-glucuronidase (GUS) assays

Seeds containing *ATH1p:GUS* reporter genes were germinated on agar plates containing phosphinothricin (10 mg per litre) for selection. For short-term treatments, 10-day-old seedlings or inflorescences from 6-week-old flowering plants were immersed in Mock (0.02% Silwett L-77) or DEX (30 µm and 0.02% Silwett L-77) solutions for 4 or 24 hours. For long-term treatments, seedlings were germinated on agar plates with or without DEX (10 µm). Seedlings were transferred to soil and sprayed daily with Mock (0.02% Silwett L-77) or DEX (10 µm DEX and 0.02% Silwett L-77) solutions until maturity. GUS assays were performed as previously described (Sieburth and Meyerowitz, 1997). After colour development, samples were cleared with 70% ethanol and imaged using an AXIO imager M2 microscope (Zeiss) with an AxioCam digital camera (Zeiss).

2.5 Phenotype analyses

For quantitative analysis of meristem arrest, seedlings were germinated on agar plates under SDs, transferred to soil on Day 10, and scored for meristem arrest on Day 25. Progenies from a selfed *pny pnf/+* plant were analysed in parallel with wild-type and mutant *bop1 bop2 pny pnf*, *tga1 pny pnf, tga4 pny pnf,* and *tga1 tga4 pny pnf* plants. Quantitative analyses of inflorescence phenotypes were performed with 8-week-old plants grown under LDs. Average height, internode length, and rosette paraclade number were determined for at least twenty-four plants per genotype as previously described (Ragni et al., 2008). Lengths were measured for the first 11-13 fully expanded internodes counting acropetally (bottom to top, towards shoot apex). Paraclades were counted as the number of axillary shoots originating from the rosette. Flowering time was scored for

at least twenty-four plants per genotype by monitoring the date of apex emergence (bolting date) since *bop1 bop2* mutants initiate leaves at a reduced rate (Norberg et al., 2005). Seeds were germinated directly on soil under LDs. 3-4 seeds were sown in each cubicle of a 72-well propagation tray and thinned at the seedling stage. One healthy seedling per well was allowed to grow to maturity.

2.6 Exogenous treatments with hormone

To analyze the effect of GA on growth, 10-day-old seedlings grown under continuous light were sprayed daily with GA (100 µm GA_3, 0.02% Silwett L-77) or a Mock solution (0.02% Silwett L-77) until maturity (Hay et al., 2002). To examine the effect of JA on growth, 10-day-old seedlings grown under LDs were sprayed daily with MeJA (100 µm MeJA, 0.02% Silwett L-77) or a Mock solution (0.02% Silwett L-77) until maturity (Canet et al., 2012). Seeds were germinated directly on soil and thinned to leave one healthy seedling per well. Plants were covered with a plastic dome for 1 hour following treatments and solutions were made fresh once a week. Flowering time was determined by scoring the date of apex emergence. Plant height was measured using a ruler. At least 24 plants per genotype were monitored.

2.7 Bimolecular fluorescence complementation (BiFC)

BiFC was used to monitor interactions between BOP1/2 and TGA transcription factors PAN, TGA1, and TGA4 *in planta*. In brief, inserts corresponding to the coding regions of *BOP2, PAN, TGA1*, and *TGA4* were cloned in-frame upstream of N-terminal or C-terminal fragments of Yellow Fluorescent Protein (YFP) in pSPYNE-$35S^{Gateway}$ or pSPYCE-$35S^{Gateway}$

vectors and expressed in Agrobacterium as previously described (Devi, 2014). Healthy green leaves of 8-week-old *Nicotiana benthamiana* (tobacco) grown under LDs were infiltrated as previously described (Sparkes et al., 2006). In brief, freshly streaked plates of Agrobacterium harbouring a BiFC construct were used to inoculate 5 ml overnight cultures in Luria Broth (LB) with selective antibiotics at 28^0C. The next day, cultures were centrifuged and the cell pellet was resuspended in 3 ml of freshly prepared infiltration buffer (10 mM $MgCl_2$, 10mM 2-[N-morpholino] ethanesulfonic acid–potassium hydroxide (MES-KOH) buffer (pH 5.6) and 150 µM of acetosyringone). To avoid co-suppression, an Agrobacterium strain expressing the viral suppressor of gene silencing p19 (Vionnet et al., 2003) was included in the infiltration mixture. The respective construct and p19 cultures were mixed in the ratio 1:1:1 and incubated at room temperature for 1 hour before infiltration of leaves. Prior to infiltration, the tobacco plants were sprayed for two days with MeJA (100 µM MeJA and 0.02% Silwett L-77) or a Mock (0.02% Silwett L-77 and 1% EtOH) solution. 1 ml needleless syringes were used for injection of culture mixtures into a flat portion of the abaxial leaf surface. After two or three days, leaf discs (2-3 mm in diameter) were mounted under a cover slip on a glass slide. Samples were viewed under a Zeiss LSM510 Meta confocal laser scanning microscope to monitor for reconstitution of YFP fluorescence as previously described (Devi, 2014).

2.8 Reverse transcriptase PCR (RT-PCR)

To carry out transcript analyses of *ath1* alleles, total RNA was isolated from 10-day-old wild-type and mutant seedlings grown on agar plates using the Trizol method as described for RT-PCR (Invitrogen). RT was performed using 1 µg of total RNA as the template and

Superscript III as the polymerase (Invitrogen). Two µl of the first-strand cDNA synthesis reaction was used as template for PCR amplification using Taq polymerase. Primers used for the detection of full-length *ATH1* transcript were ATH1-CDS-F1 and ATH1-CDS-R1. Primers used for the detection of partial *ATH1* transcript were ATH1-CDS-R1 and ATH1-R2. The ATH1-R2 primer spans an intron to prohibit amplification of genomic DNA. *GAPC* encoding cytosolic glyceraldehyde-3-phosphate dehydrogenase served as a control (Hepworth et al., 2005). 28 cycles of amplification with an annealing temperature of 59°C was used for detection of *ATH1* transcripts. 24 cycles of amplification with an annealing temperature of 58°C was used for detection of *GAPC* transcripts. An equal volume of PCR reaction plus 1 µl of SYBR Green (1:4000) was loaded onto a 0.8% agarose gel for electrophoresis prior to imaging.

2.9 Chromatin immunoprecipitation (ChIP) assay

BOP1p:BOP1-GR bop1 bop2 seedlings were germinated on agar plates containing phosphinothricin with Mock (0.04% ethanol) or DEX (10 µM). After transfer to soil, plants germinated on Mock plates where sprayed daily with Mock solution and those germinated on Dex where sprayed daily. At 4-weeks-old, leaf tissue was harvested from the respective treatment populations for analyses. ChIP was performed as described (Chakravarthy et al., 2003) using an anti-GR antibody (Santa Cruz Biotechnology). Quantification of immunoprecipitated DNA by RT-PCR was performed as previously described (Boyle et al., 2009). Primers for ChIP were as listed (Khan et al., 2015).

Table 2.1 List of primers

Description	Primer	Sequence 5' - 3'	Reference
Genotyping			
tga1-1	TGA1 Salk_028212 RP	TGAGGAATCTCCGTGTCCCCTCTGG	Devi (2014)
	TGA1 Salk_028212 LP	TTCAAAACCTGGATTCATGGTTTCC	Devi (2014)
tga4-1	TGA4 Salk_127923 RP	GAAGGTTTGAAGTTTACGAGCCTCT	Devi (2014)
	TGA4 Salk_127923 LP	GCTCTGCTGAAGTTTTCCACATTCC	Devi (2014)
Pny	BLR-Salk-RP	TTGGAATTGGAGACAAAATGTGTTA	Smith and Hake (2003)
	BLR-Salk-LP	GGAACCAAGTTCAAACTCGAATCCA	Smith and Hake (2003)
pnf	PNF-LP	TGCATGAGTTCCATATATATAGCAA	Smith et al. (2004)
	PNF-RP	TCCGATCGGTATGTGTTGTGTTCCC	Smith et al. (2004)
aos	AOS-F1	GAGAGTTATAGAAGAACCTCTCATC	This study
	CYP74A1-R1	CGAAATGTAGAGCAGCAACAGATTATACA	Park et al. (2002)
	TJRB (T-DNA)	CGGGCCTAACTTTTGGTGTGATGATGCT	Park et al. (2002)
ath1-1	ATH1TGA3.3 FWD	GCTCGGAGATAAGTCTTTGTGCAGCTA	Proveniers et al. (2007)
	35S-mini	CTGCAGCAAGACCCTTCCTCTAT	Proveniers et al. (2007)
ath1-3	ATH1-RP	GGCGGGTTTCGGATCTACATT	Gomez-Mena and Sablowski (2008)
	ATH1 LP	CCAATACCGGTTTTTCAGACATGA	Gómez-Mena and Sablowski (2008)
ath1-4	ath1-4dCAPS-F	CAAGAAGATAATCTCTATGGGATCTG	This study
	ath1-4dCAPS-R	TACGGGTGAAGGAAGTTTTGGAATAT	This study
T-DNA, left border	LBb1.3	ATTTTGCCGATTTCGGAAC	Signal.salk.edu/cgibin /T.DNA express
RT-PCR			
ATH1 Full	ATH1-CDS-F1	ATGGACAACAACAACAACAACAACACTTTTAG	This study
ATH1 Partial	ATH1-R2	CCTCCGAGTTATTATAATCACCATC	This study
	ATH1-CDS-R1	TTATTTATGCATTGCTTGGCTCATCA	This study
GAPC	GAPC-P1	TCAGACTCGAGAAAGCTGCTAC	Khan et al. (2012)
	GAPC-P2	GATCAAGTCGACCACACGG	Khan et al. (2012)
Reporter genes			
35S:ATH1	ATH1-qPCR-F1	ATACTCGCTCGATTATTCATCTCGA	Khan (2013)
	ATH1-CDS-R1	TTATTTATGCATTGCTTGGCTCATCA	Khan (2013)
2.0-kb ATH1p:GUS	ATH1prom-F3-BamH1	GGATCCGTCGGTACTATAGTTCCTAGTGT	This study
	ATH1prom-R2-NcoI	CCATGGCCATTGGGTTTCTATGAAACTGCA	This study
3.3-kb ATH1p:GUS	ATH1prom-F1-BamH1	GGATCCTCAGCAGAGAAGCCTATAAGTTC	This study
	ATH1prom-R2-NcoI	CCATGGCCATTGGGTTTCTATGAAACTGCA	This study

CHAPTER 3

RESULTS

3.1 BOP1/2 interactions with ATH1 in meristem competence to flower

Previously we showed that PNY and PNF facilitate the floral transition by repressing a set of lateral organ boundary genes defined by *BOP1/2* and its downstream effectors: *ATH1* and *KNAT6* (Smith et al., 2004; Khan et al., 2015). Inactivation of *BOP1/2* and *KNAT6* fully rescue *pny pnf* defects in meristem maintenance, internode elongation, and flowering. Some loss-of-function alleles of *ATH1* also have this effect (Tabb, 2012; Khan et al., 2015) but others do not (Rutjens et al., 2009). Thus, it was important to understand the differences between these alleles and to confirm that *ATH1* functions in the same genetic pathway as *BOP1/2* as a starting point for my study.

3.1.1 Characterization of *ath1* loss-of-function alleles

ATH1 has three mutant alleles: *ath1-1* (Proveniers et al., 2007), *ath1-3* (Gómez-Mena and Sablowski, 2008), and *ath1-4* (Li et al., 2012). A previous study using the *ath1-1* allele reported that SAM arrest in triple mutants with *pny pnf* is markedly enhanced, with little or no rescue of flowering (Rutjens et al., 2009). In contrast, Paul Tabb in our lab showed that an *ath1-3* allele completely rescues *pny* or *pny pnf* defects similar to loss-of-function *bop1 bop2* and *knat6* placing these genes in the same genetic pathway (Tabb, 2012). Further evidence showed that BOP1 directly activates *ATH1* and requires its activity to exert changes in inflorescence architecture (Khan et al., 2012a; Khan, 2013). To determine the cause of *ath1* allelic differences, I mapped the precise position of mutations in *ath1-1* and *ath1-3* (T-DNA insertion mutants) and *ath1-4* (a point mutant) in relation to functional domains in the ATH1 protein (Fig. 3.1A). I further used semi-quantitative RT-PCR to check these three alleles for expression of full or partial mutant transcripts. Finally,

I crossed *ath1-1* and *ath1-4* alleles to *pny* and *pny pnf*/+ mutants to test for phenotypic rescue in comparison to *ath1-3* (Tabb, 2012). My data showed that *ath1-3* is an RNA null with a T-DNA insertion at nt +1225 of the coding region at the 5' end of the homeodomain in ATH1. In contrast, *ath1-1* contains a T-DNA insertion at nt +849 in the BELL domain of ATH1 and expresses a partial transcript. Finally, *ath1-4* which contains single nucleotide polymorphism at nt +1261 in the homeodomain creating a stop codon expresses a full length mutant transcript (Fig. 3.1AB). Only the *ath1-3* allele fully rescues *pny* and *pny pnf* mutant defects (Tabb, 2012). Double or triple mutants of *ath1-1* or *ath1-4* with *pny* and *pny pnf* are enhanced for meristem maintenance defects and flowering in *pny pnf* is only partly rescued (Fig. 3.1C-F). These data indicate that mutant transcripts in *ath1-1* and *ath1-4* alleles have a dominant negative effect. Thus, *ATH1* is confirmed to function in the same genetic pathway as *BOP1/2* based on *ath1-3* loss-of-function interactions with *pny* and *pny pnf*.

3.1.2 Overexpression of *ATH1* mimics *pny* and *pny pnf/+* defects in inflorescence architecture

Plants that overexpress *BOP1 or BOP2* including the activation tagged line *bop1-6D* are late flowering with shortened internodes and clustered fruits similar to *pny* and *pny pnf/+* mutants (Smith et al., 2004; Norberg et al., 2005; Ha et al., 2007; Khan et al., 2012b). Plants overexpressing *KNAT6* display similar *pny*-like clustering of fruits (Shi et al., 2011) consistent with the function of these genes in the same genetic pathway. To test if overexpression of *ATH1* has a similar effect, I constructed *D35S:ATH1* transgenic plants in

which the *ATH1* coding region was expressed under the control of a double 35S CaMV promoter (Fig. 3.2). Phenotypes were scored in the T1 generation. This analysis showed that 26.1% of primary transformants (n=360) were reduced in stature and showed *pny*-like clustering of fruits on the primary stem. Weak *pny*-like clustering was observed in 9.4% of transformants, medium *pny*-like clustering was observed in 11.4% of transformants, strong *pny*-like clustering was observed in 3.6% of transformants, and severe dwarfing (a *bop1-6D*–like phenotype) was observed in 1.7% of transformants (Table 3.1). These data provide additional evidence that BOP1/2 and ATH1-KNAT6 are key components of a boundary module that blocks IM activity in *pny pnf* apices. We next examined regulatory interactions between these genes in the pathway.

3.1.3 BOP1 induces expression of *ATH1p:GUS* reporter genes in leaves and inflorescences

To test if *ATH1* and/or *KNAT6* were immediate transcriptional targets of BOP1/2, Madiha used a transgenic line expressing a translational fusion of BOP1 to the steroid-binding domain of the rat glucocorticoid receptor (Lloyd et al., 1994). Treatment with dexamethasone (DEX) leads to translocation of the BOP1-GR fusion protein from the cytoplasm to the nucleus as a way of controlling post-translational activity (Lloyd et al., 1994). This method showed that *ATH1* is a direct transcriptional target of BOP1 whereas activation of *KNAT6* is indirect (Khan, 2013).

To confirm that the *ATH1* promoter is responsive to BOP1 induction, 2.0-kb and 3.3-kb fragments of the upstream genomic region of *ATH1* including the start site of translation

were fused in-frame to the coding region of a GUS reporter gene (Jefferson et al., 1987). These reporters were expressed in a *D35S:BOP1-GR* transgenic background to permit downstream analysis of *ATH1* expression in response to DEX-induction of BOP1 activity. GUS activity in leaves and inflorescences was monitored in the T1 generation to confirm that fusions were active (data not shown). Three representative lines for each promoter were chosen for further analysis in T2 and T3 generations. Consistent with previous reports (Proveniers et al., 2007; Gómez-Mena and Sablowski, 2008) reporters were expressed in shoot apices, in leaf vasculature and the petiole base, at the base of floral organs before and after abscission, and weakly in the stem (Fig. 3.3). These data confirmed that the *ATH1* promoter is active in seedlings and the base of floral organs in a pattern that overlaps with *BOP1* and *BOP2* expression domains (McKim et al., 2008; Xu et al., 2010; Khan et al., 2012b).

Overexpression of BOP1 in wild-type plants generates a dwarf phenotype due to restricted elongation of internodes in the inflorescence (Norberg et al., 2005; Khan et al., 2012b). To confirm that the BOP1-GR fusion was active in 2.0 and 3.3-kb reporter lines, I compared the average height of plants after treatments with Mock (0.02% Silwett L-77) or DEX (30 μM 0.02% Silwett L-77) solutions for six weeks (Fig. 3.4). Two independent homozygous lines were used for each reporter line. Twelve plants were measured for each treatment group. 2-kb *ATH1p:GUS D35S:BOP1-GR* lines treated with Mock solution (23.8 $\pm$ 1.0 cm and 21.1 $\pm$ 1.6 cm) were similar in height to Col wild-type plants (18.3 ± 0.9 cm and 19.3 ± 1.2 cm). Likewise, 3.3-kb *ATH1p:GUS D35S:BOP1-GR* lines treated with Mock solution (25.4 ± 1 cm and 23.6 $\pm$ 1.9 cm) were similar in height to Col WT (24.7 $\pm$

1.1 cm and 18.2 ± 1.1 cm). In all cases, DEX-treated 2-kb *ATH1p:GUS D35S:BOP1-GR* (10.3 ± 0.5 cm and 9.4 ± 0.4 cm) and 3.3-kb *ATH1p:GUS D35S:BOP1-GR* (13.7 ± 0.7 cm and 9.2 ± 0.6 cm) lines were significantly shorter than control plants. These data confirm activity of the BOP1-GR fusion protein in reporter lines.

Inducible expression of 2.0-kb and 3.3-kb *ATH1p*:GUS in *D35S:BOP1-GR* lines was monitored in T2 lines following short-term (24 hours) or long-term (six weeks) treatment with DEX. Promoter activity was monitored by GUS staining after incubation of inflorescence apices in Mock (0.02% Silwett L-77) or DEX (30 µM and 0.02% Silwett L-77) solutions for 24 hours or after daily treatments with Mock (0.02% Silwett L-77) or DEX (10 µM and 0.02% Silwett L-77) solutions for six weeks. Three independent lines were analyzed for each promoter construct. At least ten plants were analyzed in each treatment group. Comparison of Mock and DEX panels shows that expression is upregulated in the stems and floral organs of DEX-induced lines for both promoter constructs (Fig. 3.5). These data show that both 2.0-kb and 3.3-kb promoter lengths are responsive to BOP1 induction.

3.1.4 Identification of the genomic region responsible for BOP1 induction of *ATH1* expression

Given that BOP1-GR binds directly to *ATH1* to activate its expression, a rapid increase in GUS activity is expected after induction with DEX (Jun et al., 2010). To test for rapid induction, seedlings and inflorescences of 2-kb and 3.3-kb *ATH1p:GUS D35S:BOP1-GR* transgenic lines were stained after 2 and 4 hours of treatment with Mock or DEX solutions.

The experiment was carried out in the T3 generation using homozygous lines. After 4 hours of DEX treatment, GUS activity was visibly enhanced in leaves, flowers, and internodes relative to Mock-treated controls for both promoter lengths (Fig. 3.6A-H). These data are consistent with the model that BOP1 activates transcription by binding to elements located within a minimal 2.0-kb fragment of the *ATH1* promoter.

BTB-ankyrin proteins including BOP1/2 lack a recognizable DNA-binding domain and interact with TGA bZIP binding factors for recruitment to DNA (Després et al., 2000; Hepworth et al., 2005; Xu et al., 2010; Khan et al., 2014). Inspection of the *ATH1* promoter identified numerous motifs that match or closely match consensus binding sites for TGA factors. We collaborated with Charles Després at Brock University to determine if any of these sites were biologically relevant. To test if BOP1 directly associates with the *ATH1* promoter, we performed chromatin immunoprecipitation (ChIP) assay using leaves harvested from *BOP1p:BOP1-GR bop1 bop2* flowering plants and an anti-GR antibody. ChIP assays were performed using 8 sets of primers spanning 2178 base pairs of genomic sequence upstream of the *ATH1* transcription start site based on regions enriched in TGA bZIP binding sites (Fig. 3.6I). Quantitative analysis by qRT-PCR revealed at least one position in the *ATH1* promoter (Site IV) showing a reproducible 1.77-fold enrichment of BOP1 protein in DEX-treated plants (Fig. 3.6J). ChIP assays performed using the Mock control showed no obvious enrichment at this position nor at the control *UBQ5* genomic region. Site IV (nt –2686 to -2577) is located approximately 1515 base pairs upstream of the *ATH1* transcription start site and found within the 3.3-kb *ATH1p:GUS* construct that is responsive to BOP1 induction in leaves and inflorescences (Fig. 3.2). Site VII (nt –1529 to

-1416) was identified as a second potential binding site at lower confidence located within the 2.0-kb promoter interval. Taken together, these data support that BOP1 directly associates with the *ATH1* promoter *in vivo* to regulate its transcription.

3.2 Role of clade I TGA factors as co-factors for BOP1/2 in regulation of internode elongation and flowering

TGA bZIP transcription factors mediate diverse functions in plant development and defense (Jakoby et al., 2002; Gatz, 2013). In Arabidopsis, five clades of TGA factors have been classified based on functional and structural features (Jakoby et al., 2002; Gatz, 2013). Clade I members TGA1 and TGA4 have dual roles in defense and leaf development but have not previously been linked to BOP1/2 activity (Després et al., 2003; Kesarwani et al., 2007; Song et al., 2008; Shearer et al., 2012; Devi, 2014). Discovery of *TGA1* and *TGA4* expression at lateral organ boundaries in a pattern that overlaps with BOP1 and BOP2 (Devi, 2014) prompted us to test if these factors mediate BOP1/2 activity in repression of internode elongation and flowering in *pny* and *pny pnf* mutants.

3.2.1 Inactivation of *TGA1* and *TGA4* rescues *pny* defects in inflorescence architecture

Loss-of-function mutations in *pny* delay flowering and lead to changes in inflorescence patterning that include partial loss of apical dominance and clustering of fruits caused by irregular elongation of internodes on the primary stem (Byrne et al., 2003; Smith and Hake, 2003). These defects are caused by misexpression of *BOP1/2*, *KNAT6*, and *ATH1* in the stem cortex of *pny* mutants (Ragni et al., 2008; Khan et al., 2012a; Khan et al., 2012b).

Compatible with this, inactivation of *BOP1/2*, *KNAT6*, and *ATH1* fully rescue *pny* defects in inflorescence architecture (Khan et al., 2012a; Khan et al., 2012b). To test if TGA1 and TGA4 are members of the same genetic pathways, we constructed *tga1 pny, tga4 pny,* and *tga1 tga4 pny* mutants and analyzed the resulting phenotypes. Fig. 3.7 shows inactivation of *TGA1* alone partially rescues *pny* clustering of fruits on the primary stem whereas inactivation of *TGA4* has no such effect. However, inactivation of *TGA1* and *TGA4* together fully rescues *pny* inflorescence resulting in a pattern similar to wild-type (Fig. 3.7ABF). Quantitative phenotypic analysis was carried out on at least 12 plants per genotype to further monitor this rescue, by measuring average height (data not shown), internode length, and number of rosette paraclades for wild-type and mutants. These analyses confirmed that loss-of-function *tga1 tga4* restore the number of rosette paraclades to wild-type. Whereas *pny* mutants have a significant number of internodes in the 1-5 mm range, the distribution in *tga1 tga4 pny* mutants was similar to wild-type. These data support that BOP1/2 and clade I TGA factors function in the same genetic pathway to control inflorescence architecture.

3.2.1 Inactivation of Clade I TGA factors rescues *pny pnf* defects in SAM maintenance and flowering

Meristems lacking PNY and PNF frequently terminate after the production of 3-5 leaves with development resuming from meristems in the axils of rosette leaves (Smith et al., 2004; Rutjens et al., 2009). Meristems in *pny pnf* are competent to produce leaves but unable to support the production of internodes or flowers (Smith et al., 2004).

Inactivation of *BOP1/2*, *KNAT6*, and *ATH1* restores normal development in *pny pnf* mutants (Tabb, 2012; Khan et al., 2015). To test if *TGA1* and *TGA4* are members of this same genetic pathway, we constructed *tga1 pny pnf, tga4 pny pnf,* and *tga1 tga4 pny pnf* mutants and analyzed the resulting phenotypes. Fig. 3.8 shows that inactivation of *TGA1* and *TGA4* alone or in combination rescues *pny pnf* defects in SAM maintenance. Fig. 3.9 shows that inactivation of *TGA1* and *TGA4* also rescue flowering. Inflorescence architecture and flowering in the *tga1 tga4 pny pnf* quadruple mutant is fully rescued—plants look wild-type. Flowering time in the *tga4 pny pnf* triple mutant is similar to wild-type but *pny*-like clusters are observed in the inflorescence indicating incomplete rescue of internode elongation. Flowering time in the *tga1 pny pnf* triple mutant is significantly delayed related to wild-type control plants and fruits are clustered. Thus, inactivation of *TGA1* and *TGA4* in *pny pnf* restores flowering but their functions are not completely identical.

3.2.2 Bimolecular fluorescence complementation (BiFC) fails to show a direct interaction of BOP2 with clade I TGA factors

Overall, my data show that BOP1/2 and clade I TGA factors function in the same genetic pathway to control flowering. This prompted us to test for protein-protein interactions *in vivo* using BiFC. Only the BOP2 constructs were analyzed in my study. Previously, BOP1 interaction with either TGA1/4 were analysed (Devi, 2013). BOP2 or TGA proteins were fused at the C-terminus in-frame to the N-terminal or C-terminal halves of YFP (nYFP or cYFP). Agrobacterium containing these constructs were mixed in pairs and infiltrated into

tobacco leaves for transient expression in epidermal cells. Interacting proteins that bring the two halves of YFP back together restore fluorescence that can be monitored by confocal microscopy.

Preliminary BiFC experiments showed that BOP1/2 do not constitutively interact with TGA1 or TGA4 suggesting that post-translational regulation is a possibility (Devi, 2014). In plant defense, salicylic acid triggers a conformational change in NPR1 and converts regulatory cysteine residues in TGA1 and TGA4 to a reduced state allowing an interaction to take place (Després et al., 2003). BOP1/2 gain-of-function lines have increased JA content and enhanced MeJA-induced resistance to bacterial pathogen (Canet et al., 2012; Khan et al., 2015). We hypothesized that MeJA promotes BOP1/2 interaction with TGA1 and TGA4. BiFC was carried out in plants sprayed with MeJA (100 µM, 0.02% Silwett L-77) or Mock (Silwett L-77) solutions to test this idea. Fig. 3.10 shows that no fluorescence was detected when cYFP alone or BOP2-nYFP + cYFP combinations were expressed in plants. *35S:YFP* served as a positive control with yellow fluorescence detected in the cytoplasm and nucleus. Yellow fluorescence in the nucleus was readily detected for BOP2-PAN interactions but no interaction of BOP2 with TGA1 or TGA4 was detected in the presence or absence of MeJA.

3.3 Investigating antagonism between jasmonic acid and gibberellin as a mechanism for repression of flowering in *pny pnf* mutants

Presumably, the BOP1/2 module activates a set of genes that repress meristem competence to flower. To identify these genes, microarray was used to analyze the

transcriptome of *bop1-6D* versus wild-type internodes (Khan et al., 2015). Gene Ontology (GO) analysis of differentially regulated genes revealed significant enrichment of terms associated with response to biotic and abiotic stress stimuli (Appendix I). Response to jasmonic acid (JA) stimulus (GO:009753) was at the top of the list, but other hormone pathways associated with stress showed similar enrichment. In descending order these were response to salicylic acid stimulus (GO:0009751), response to ethylene stimulus (GO:009723) and response to abscisic acid stimulus (GO:0099737). These data suggest that *bop1-6D* plants have heighted expression of stress-related genes. Trade-offs between plant defense and plant growth via repression of GA biosynthetic pathways are well-established in the recent literature (Navarro et al., 2008; Wild et al., 2012; Yang et al., 2012; Wild and Achard, 2013) so we further explored this mechanism.

3.3.1 Exogenous GA fails to rescue internode elongation and flowering in *pny pnf* mutant and transgenic plants overexpressing BOP1

Transcriptional profiling of *pny pnf* and *bop1-6D* apices was consistent with the notion that GA deficiency was a potential factor in repression of flowering (Khan et al., 2015). I therefore tested if exogenous application of GA rescues internode elongation or flowering in these similar mutants. Wild-type and mutants grown under continuous light were sprayed daily with 100 µM GA_3 or a Mock solution until maturity. Fig. 3.11 shows that exogenous treatment with GA_3 neither rescues flowering in *pny pnf* nor enhance internode elongation in *bop1-6D*, although this mutant flowered 4 days earlier than Mock-treated control plants. A similar acceleration was seen for treatment of wild-type

control plants. These data indicate that deficiency in GA alone does not account for defects in internode elongation and flowering in *pny pnf* and *bop1-6D* mutants.

3.3.2 Effect of loss and gain of JA content on flowering and internode elongation in wild-type and mutants

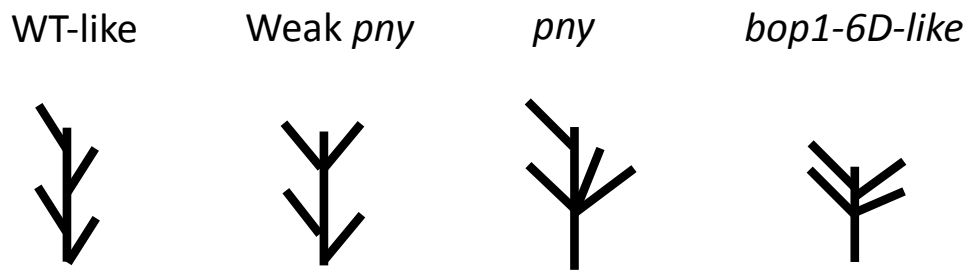
Madiha in our lab showed that up-regulation of stress markers in *BOP1* overexpressing lines correlates with an increase in the expression of JA biosynthetic genes and JA content in *pny pnf* apices and *bop1-6D* mutants. Plants that overexpress JA are stunted in growth of roots, leaves, and stems (Ellis et al., 2002; Cipollini, 2005; Bonaventure et al., 2007; Hyun et al., 2008; Zhang and Turner, 2008; Heinrich et al., 2012) but little is known about the effect of MeJA on flowering. To address this question, MeJA was applied to wild-type and *pny* plants grown under LDs (Fig. 3.12A-G). Plants of both genotypes treated with MeJA developed a compact rosette of small dark green leaves, similar to *bop1-6D* mutants (Fig. 10A-C). Wild-type plants treated with MeJA showed partial loss of apical dominance similar to *pny* mutants (Fig. 3.12D). Plants in both treatment populations were late flowering with short internodes relative to Mock-treated control plants (Fig. 10D-F) and similar to *pny pnf/+* mutants. Organ fusions or clusters were not observed. In both wild-type and *pny* populations, a small subset of plants developed a disordered rosette phenotype similar to *pny pnf* mutants and were non-flowering after 10 weeks (data not shown). No such defects were observed in Mock-treated control plants. Thus, treatment of wild-type plants with exogenous MeJA mimics the phenotype of *bop1-6D* and *pny* or *pny pnf/+* plants.

In parallel, we tested if reducing JA content rescues internode elongation or flowering in *pny pnf* and/or *bop1-6D* mutants. These lines were crossed to an *aos* mutant that is defective in allene oxide synthase which blocks the first step in the JA biosynthetic pathway (Park et al., 2002). No rescue of SAM maintenance, internode elongation, or flowering was observed in *pny pnf aos* triple mutants. Plants remained non-flowering even with the addition of exogenous GA_3 (Fig. 3.12H and data not shown). However, quantitative analysis of *bop1-6D aos* double mutants revealed a small but significant ($p \leq 0.0001$) increase in flowering-time (+1.8 days) and plant height (+1.5 cm) compared to *bop1-6D* siblings in a segregating population (Fig. 3.12IJ). These data provide evidence that modulation of growth by JA is a contributing factors in conditioning *bop1-6D* and *pny pnf* phenotypic defects.

Table 3.1 Quantification of *D35S:ATH1* overexpression phenotypes

A *D35S:ATH1* transgene was expressed in wild-type plants. Phenotypes were scored in the T1 generation. Five phenotypic categories were observed consistent with the known spectrum of BOP1/2 gain-of-function phenotypes including short stature and fruit clustering (see table). Stock number for Agrobacterium strain in the Hepworth lab glycerol collection, B573.

Phenotype	Height (cm)	Number of clusters	Number of transformants	Percent of total (n=360)
WT-like	>10	0	266	73.9
Weak *pny*	>10	<5	34	9.4
Medium *pny*	5–10	<10	41	11.4
Strong *pny*	5–10	>10	13	3.6
bop1-6D-like	<5	>10	6	1.7



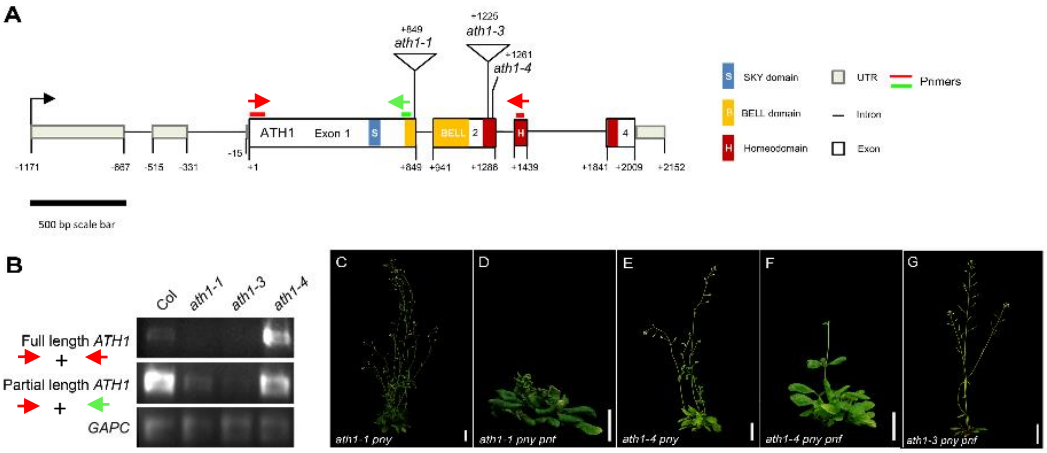
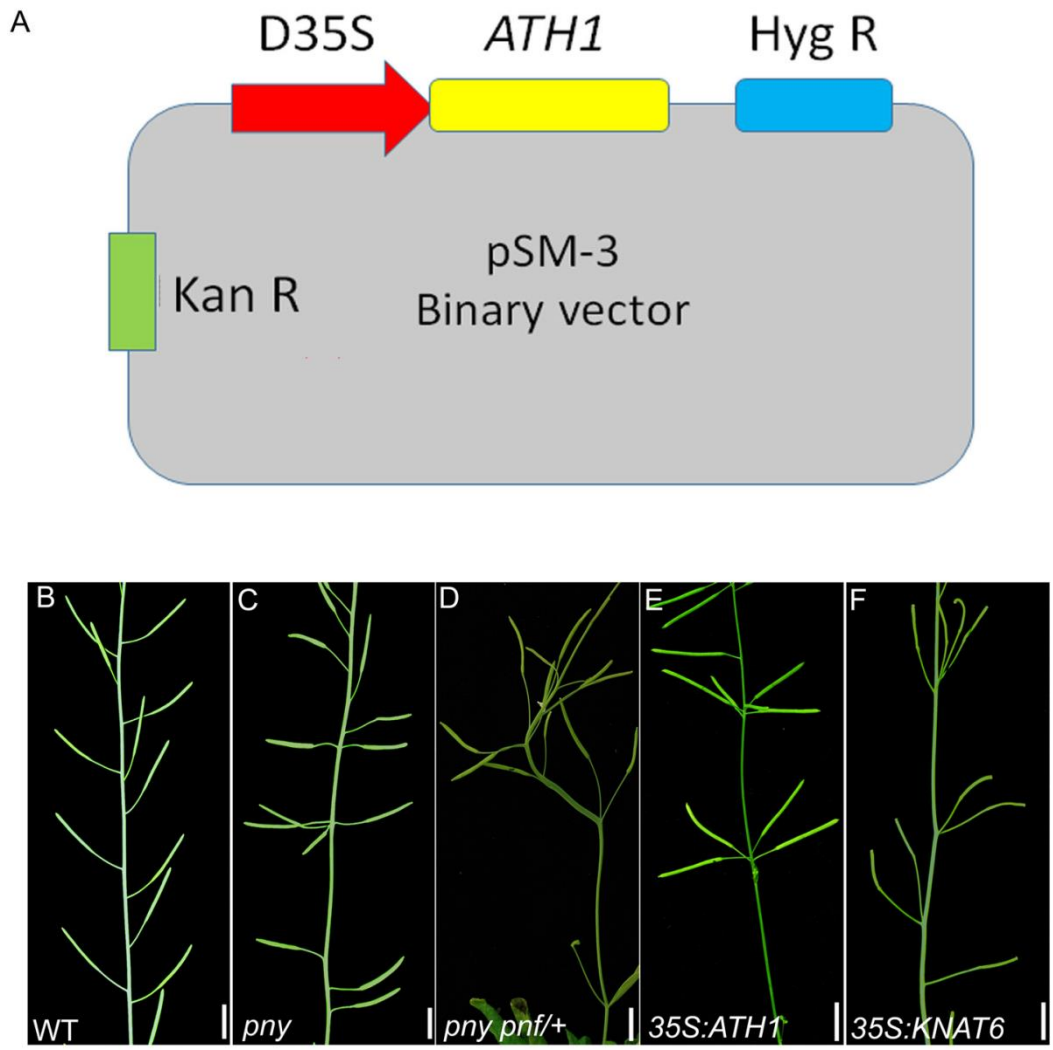


Figure 3.1 *ATH1* map and characterization of mutant alleles

(A) Map of the *ATH1* genomic region drawn to scale. Translation start site (+1). T-DNA insertion points in *ath1-1* and *ath1-3* were determined by sequencing. Polymorphism in *ath1-4* determined by Li et al. (2012). (B) Analysis of *ATH1* full and partial transcript production in wild type and mutants by semi-quantitative RT-PCR. Position of primers, (Red) Full length ~ 1422bp, (Green) Partial length ~775bp primers. Only *ath1-3* is a null allele. (C) *ath1-1 pny* mutant. Plants have a disordered rosette similar to *pny pnf* mutants but eventually flower. Internode clustering is partially rescued. Plants are semi-sterile due to carpel defects. (D) *ath1-1 pny pnf* mutant. Plants eventually produce an internode with leafy structures. (E) *ath1-4 pny* mutant; similar to *ath1-1 pny*. (F) *ath1-4 pny pnf* mutant; similar to *ath1-1 pny pnf.* Most plants produce one or more internodes. Leafy structures are typically formed in place of flowers. Occasional plants form an infertile flower. (G) *ath1-3 pny pnf* mutant completely rescues flowering. Scale bars, 1.5 cm.

Figure 3.2 Overexpression of *ATH1* mimics *pny* and *pny pnf/+* mutant phenotypes

(A) Schematic representation of *D35S:ATH1* constructs used for overexpression analyses. The plasmid background was the binary vector pSM-3 conferring kanamycin resistance (Kan R) in bacteria and hygromycin resistance (Hyg R) in plants. *ATH1* coding region was expressed under the control of a double *35S* (*D35S*) viral promoter. (B) Wild type inflorescence. (C) *pny* mutant; clustering of fruits and irregular internode lengths. (D) *pny pnf/+* hemizygous inflorescence; severe clustering of fruits and stem-pedicel fusions. (E) *35S:ATH1* transformant; mimics a *pny* phenotype. 26.1% of primary transformants (n=360) showed a reduction in plant height and fruit clustering (see Table 3.1). (F) *35S:KNAT6* transformant; mimics a *pny* phenotype similar to *35S:ATH1*. Scale bar, 1.5 cm.


A
D35S
ATH1
Hyg R
Kan R
pSM-3
Binary vector
B
C
D
E
F
WT
pny
pny pnf/+
35S:ATH1
35S:KNAT6

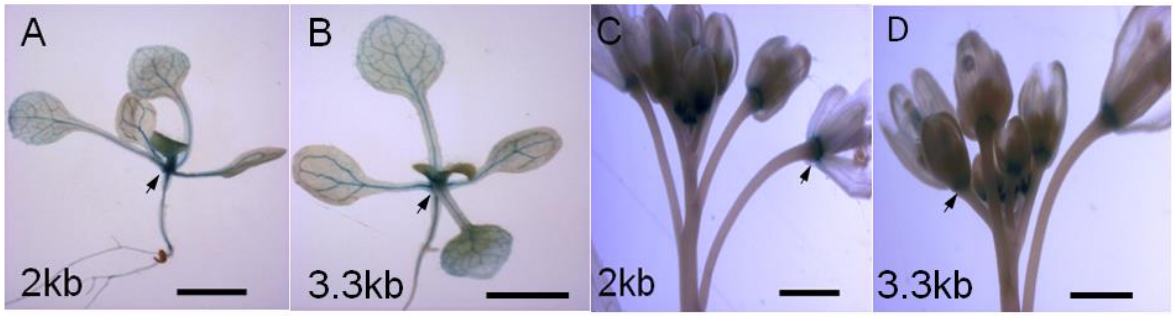


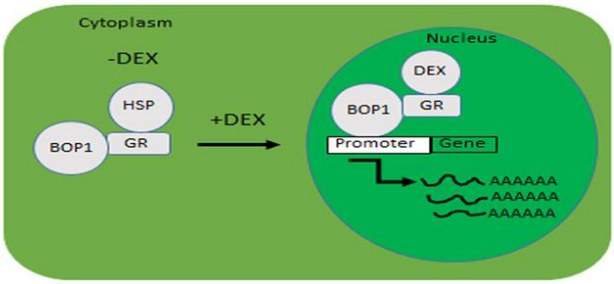
Figure 3.3 Expression pattern of *ATH1p:GUS* in seedlings and inflorescences

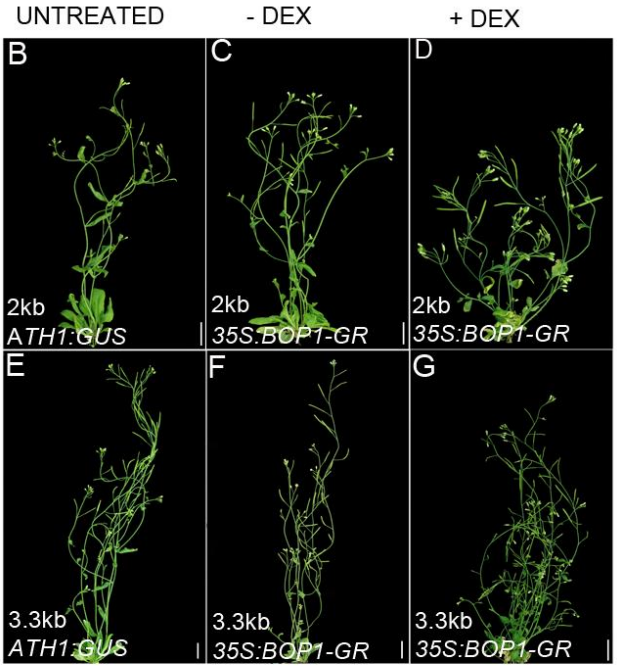
(A-B) Representative expression pattern of 2-kb and 3.3-kb *ATH1p:GUS* reporters in 10-day-old-seedlings in young leaves and base of older leaves, leaf vasculature, and hypocotyl. (C-D) Representative expression pattern of 2-kb and 3.3-kb *ATH1p:GUS* reporters in 6-week-old inflorescences at the base of floral organs and weakly in the stem at nodes and in the vasculature. Reporters were expressed in a *D35S:BOP1-GR* background. Staining was conducted in the T2 generation (n>15 plants per genotype). Expression pattern for *ATH1p:GUS* overlaps with the expression pattern of *BOP1p:GUS* and *BOP2p:GUS* at lateral organ boundaries (McKim et al., 2008; Khan et al., 2012b). Scale bars, 1 cm.

Figure 3.4 The BOP1-GR fusion protein is functional *in vivo*

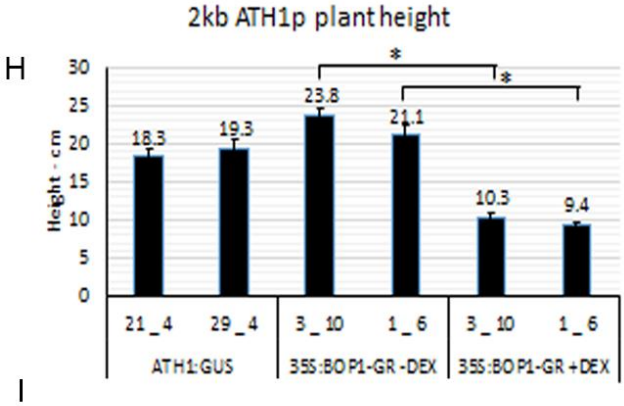
(A) Schematic of steroid induction system used for post-translational control of BOP1 activity. The *BOP1* coding region was fused in-frame to sequence encoding the steroid binding domain of the rat glucocorticoid receptor (GR) (Lloyd et al., 1994). An endogenous chaperone protein HSP90 binds to the GR portion of the fusion protein and prevents nuclear localization. Addition of DEX displaces HSP90 resulting in rapid import of the fusion protein to the nucleus and activation of target genes. Scale bars, 2 cm. (B – G) Phenotype of 6-week-old wild-type (WT) and 2.0-kb or 3.3-kb *ATH1p:GUS D35S:BOP1:GR* plants treated daily with a solution of Mock or DEX (10 µm). (H-I) Quantitative analyses of plant height. Inflorescence height was measured for two independent homozygous lines for each genotype. In all cases, DEX-treated *D35S:BOP1-GR* containing reporter lines were shorter than Mock-treated and WT control plants. Twelve plants per genotype were analyzed. An asterisk (*) indicate a significant difference ($p<0.01$) according to a student's t-test. Error bars, S.E.M.

A

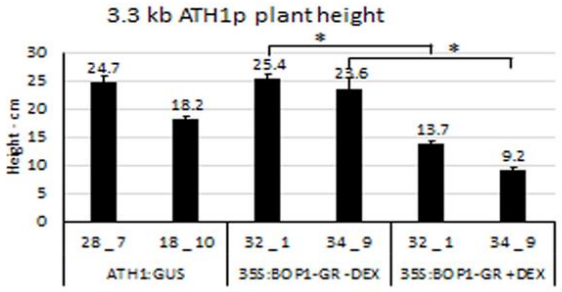

Cytoplasm
-DEX
HSP
BOP1
GR
+DEX
Nucleus
DEX
GR
BOP1
Promoter
Gene
AAAAAA
AAAAAA
AAAAAA


UNTREATED
- DEX
+ DEX
B
2kb
ATH1:GUS
C
2kb
35S:BOP1-GR
D
2kb
35S:BOP1-GR
E
3.3kb
ATH1:GUS
F
3.3kb
35S:BOP1-GR
G
3.3kb
35S:BOP1-GR

H


2kb ATH1p plant height
Height - cm
30
25
20
15
10
5
0
18.3
19.3
23.8
21.1
10.3
9.4
*
*
21_4
29_4
3_10
1_6
3_10
1_6
ATH1:GUS
35S:BOP1-GR -DEX
35S:BOP1-GR +DEX

I


3.3 kb ATH1p plant height
Height - cm
30
25
20
15
10
5
0
24.7
18.2
25.4
23.6
13.7
9.2
*
*
28_7
18_10
32_1
34_9
32_1
34_9
ATH1:GUS
35S:BOP1-GR -DEX
35S:BOP1-GR +DEX

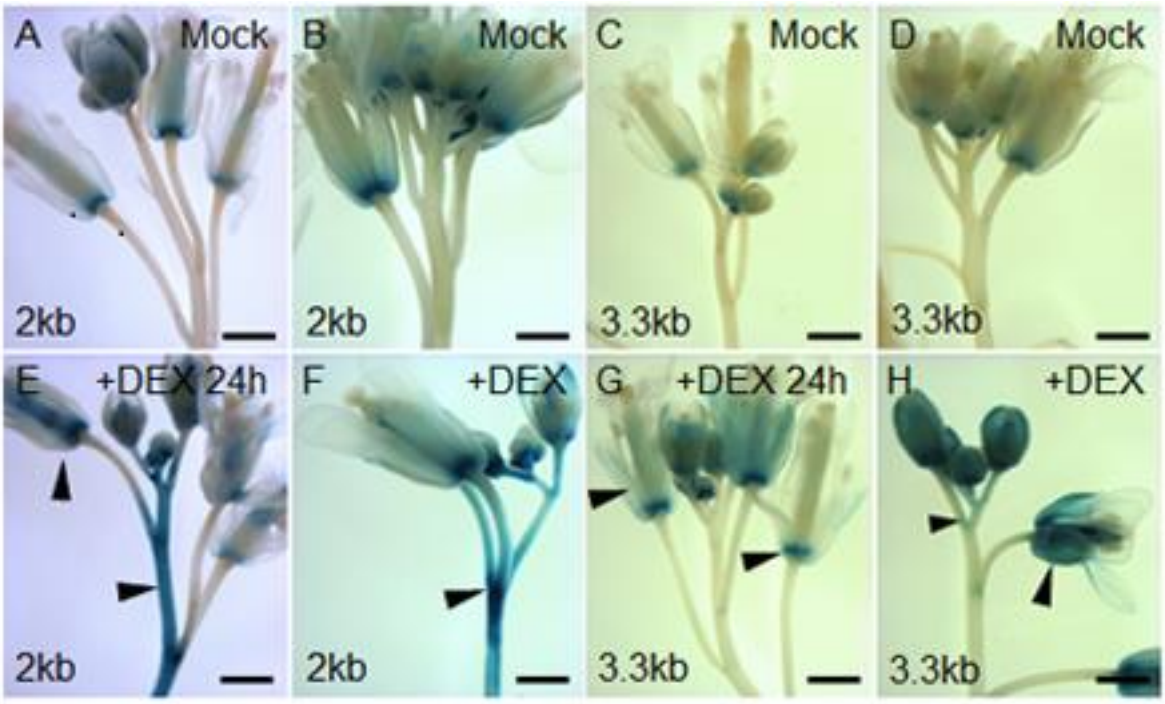


Figure 3.5 DEX-inducible expression of *ATH1p:GUS* reporter genes

Representative expression patterns are shown for *D35S:BOP1-GR* plants containing 2-kb (A, B, E, F) or 3.3-kb (C, D, G, H) *ATH1p:GUS* reporter genes in the T2 generation. Promoter activity was monitored by GUS staining after incubation of inflorescences in Mock or 30 µm DEX solutions for 24 hours (A, C, E, G) or after daily treatment of plants with Mock or 10 µm DEX solutions for six weeks (B, F, D, H). Comparison of Mock (A-D) and DEX (E-H) panels shows that expression is upregulated in the stems and floral parts of DEX-induced lines for both promoter constructs. Arrows in panels indicate expansion or enrichment of *ATH1* expression after DEX treatment. Three independent transformants were analyzed for each promoter constructs. At least ten plants were analyzed for each line. Scale bars, 1 mm.

Figure 3.6 Identification of genomic region responsible for *ATH1* induction by BOP1

(A-H) Functional characterization of the *ATH1* regulatory region. Representative expression patterns are shown for *D35S:BOP1-GR* plants containing 2-kb (A, C, E, G) or 3.3-kb (B, D, F, H) *ATH1p:GUS* reporter genes as diagrammed in (I). Promoter activity was monitored by GUS staining after incubation of tissue for 4 hours in Mock or 30 µm DEX solutions. Comparison of Mock (A-D) and DEX (E-H) panels shows that expression is upregulated in the leaves, flowers, and the stem of DEX-induced lines for both promoter constructs. Scale bars, 1 mm. (I) Map of the *ATH1* promoter and 5' untranslated region. Closed arrowheads mark the 5' end of genomic fragments used in 2-kb and 3.3-kb *ATH1p:GUS* reporter genes. Predicted consensus binding sites for TGA bZIP factors (Schindler et al., 1992; Izawa et al., 1993; Fode et al., 2008) are shown relative to fragments amplified by qRT-PCR after ChIP to test for BOP1 localization (horizontal bars). Sites in red (IV and VII) contain A-boxes and show enrichment for BOP1: site IV (nt –2686 to -2577) at ~1515 base pairs and Site VII (nt –1529 to -1416) at ~400 base pairs upstream of the *ATH1* transcription start site. (J) Immunoprecipitated DNA enrichment was quantified by qRT-PCR. Anti-GR ChIP was performed using leaves from Mock and DEX-treated *35S:BOP1-GR bop1 bop2 plants*. Fold-enrichment at sites IV and VII is presented as the ratio of DEX versus Mock transcript levels after normalization to the unrelated *UBQ5* control sequence. Three biological replicates were quantified to show enrichment at Site IV. One biological replicate was quantified to show enrichment at Site VII. Three technical replicates were performed for each. Error bars, S.D.

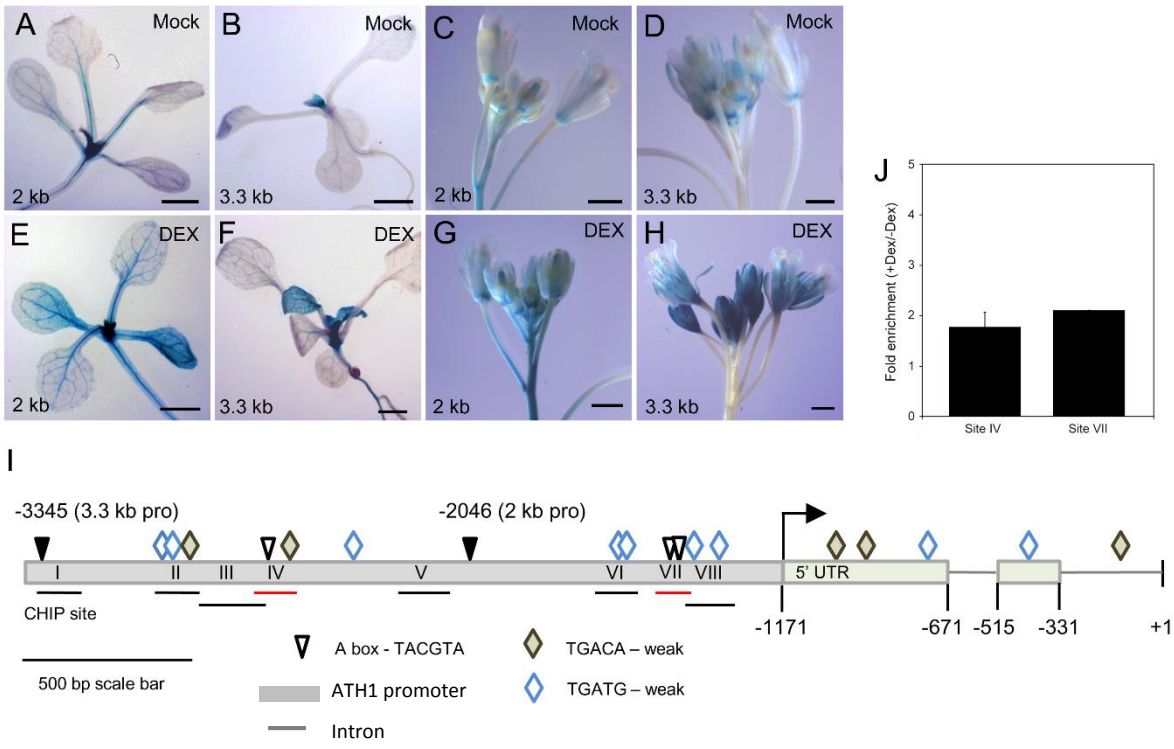
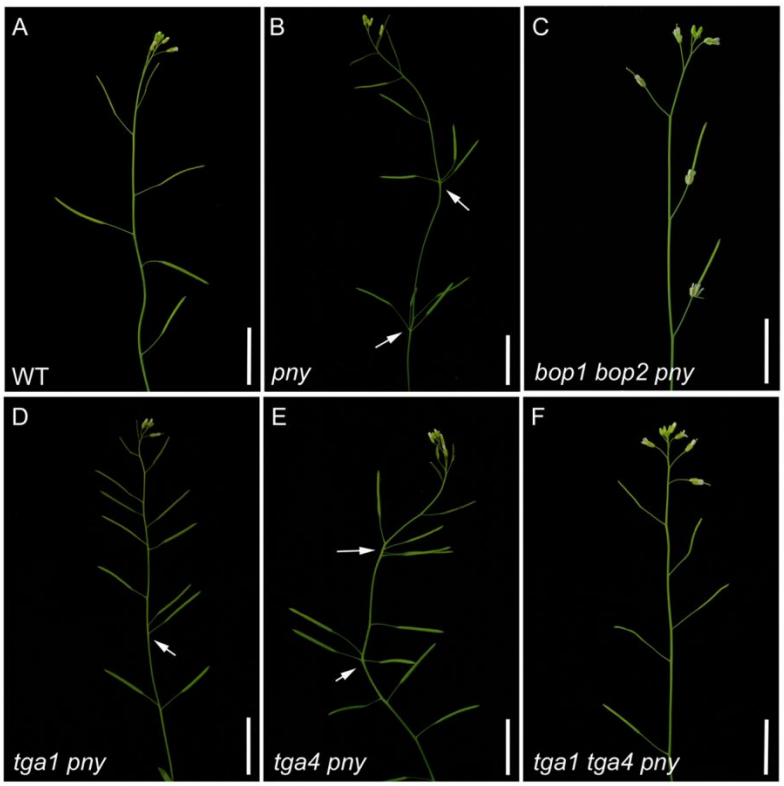
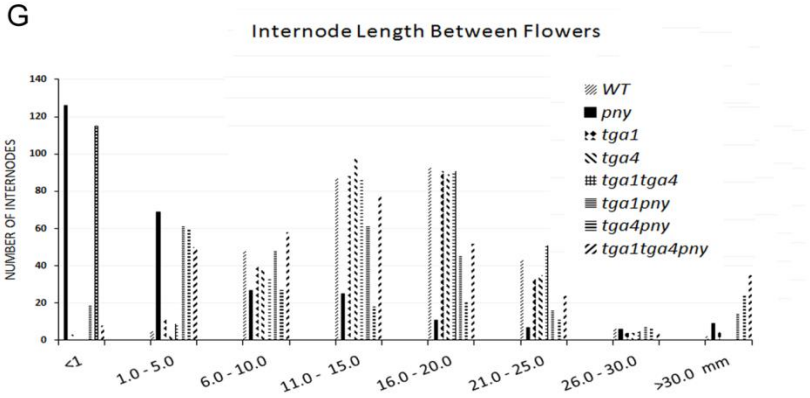

A
Mock
2 kb
B
Mock
3.3 kb
C
Mock
2 kb
D
Mock
3.3 kb
E
DEX
2 kb
F
DEX
3.3 kb
G
DEX
2 kb
H
DEX
3.3 kb
J
Fold enrichment (+Dex/-Dex)
0
1
2
3
4
5
Site IV
Site VII
I
-3345 (3.3 kb pro)
-2046 (2 kb pro)
I
II
III
IV
V
VI
VII
VIII
5' UTR
CHIP site
-1171
-671
-515
-331
+1
500 bp scale bar
A box - TACGTA
TGACA – weak
ATH1 promoter
TGATG – weak
Intron

Figure 3.7 Inactivation of *TGA1* and *TGA4* rescues *pny* inflorescence defects

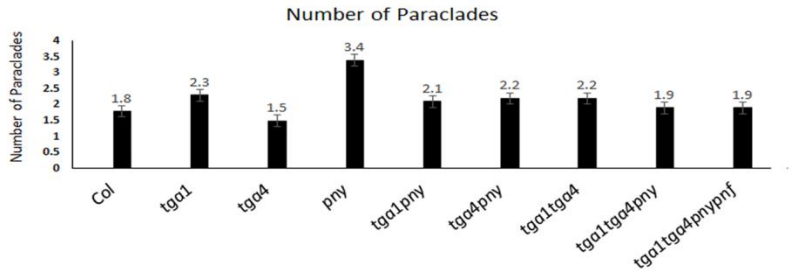
(A-E) Representative 6-week-old plants are shown. (A) Wild-type control. (B) *pny* mutant; irregular internode elongation seen as clusters of fruits on the primary stem (arrows). (C) *tga1 pny* mutant; partial rescue of clustering (arrows). (D) *tga4 pny pnf* mutant; little or no rescue of clustering (arrows). (E) *tga1 tga4 pny pnf* mutant; complete rescue of clustering; similar to wild-type. Scale bars, 1.5 cm. (F-G) Quantitative analysis of *pny* phenotypic rescue by *tga1* and *tga4* conducted on 8-week-old plants. Twenty-four plants per genotype were measured. (F) Distribution of internode lengths between successive siliques on the primary stem for plants grown in continuous light. Internodes between the first and 11^{th} siliques (counting acropetally) were measured. Distribution of internode lengths in the *tga1 tga4 pny pnf* quadruple mutant is similar to wild-type. (G) Average number of rosette paraclades in wild-type and mutants for plants grown under LDs. Inactivation of *TGA1* and *TGA4* restores apical dominance in *pny* mutants. Error bars, S.E.M.


A
WT
B
pny
C
bop1 bop2 pny
D
tga1 pny
E
tga4 pny
F
tga1 tga4 pny

G


Internode Length Between Flowers
NUMBER OF INTERNODES
140
120
100
80
60
40
20
0
WT
pny
tga1
tga4
tga1tga4
tga1pny
tga4pny
tga1tga4pny
<1
1.0 - 5.0
6.0 - 10.0
11.0 - 15.0
16.0 - 20.0
21.0 - 25.0
26.0 - 30.0
>30.0 mm

H


Number of Paraclades
Number of Paraclades
4
3.5
3
2.5
2
1.5
1
0.5
0
1.8
2.3
1.5
3.4
2.1
2.2
2.2
1.9
1.9
Col
tga1
tga4
pny
tga1pny
tga4pny
tga1tga4
tga1tga4pny
tga1tga4pnypnf

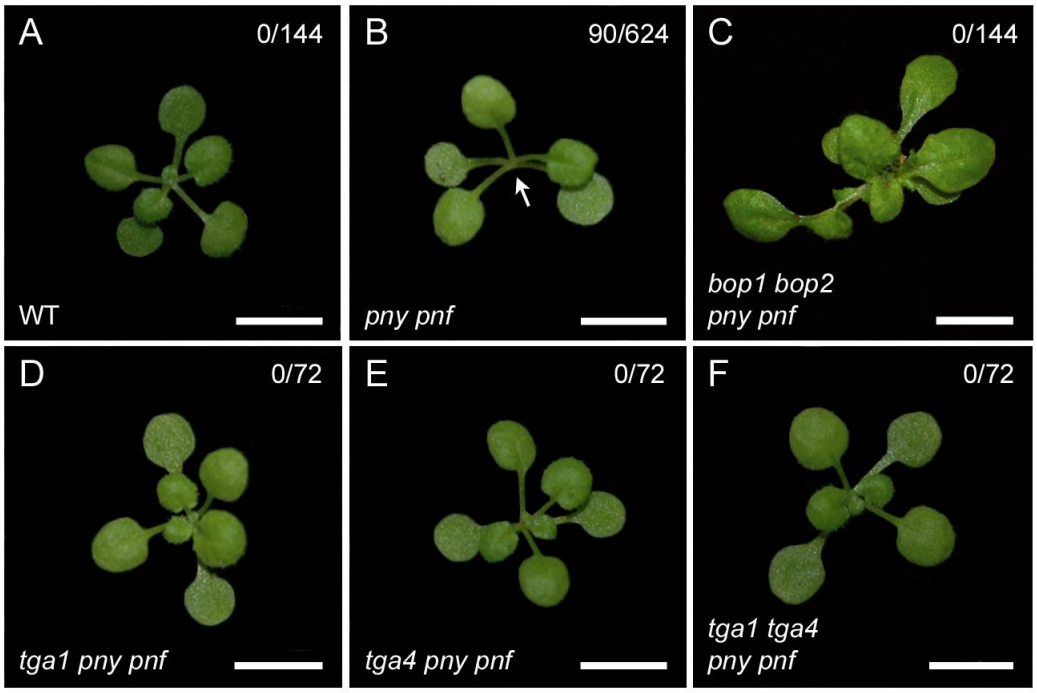


Figure 3.8. Inactivation of *TGA1* and *TGA4* rescues *pny pnf* SAM maintenance defects

Plants were grown under SDs. Number of plants showing a meristem arrest on Day 25 is indicated at top right of panels. (A) Col plant. The SAM produces leaves. (B) *pny pnf* mutant showing a meristem arrest. 90/156 (57.7%) of expected *pny pnf* mutants in a *pny pnf/+* segregating population (n=624) showed SAM arrest (arrow). (C) *bop1 bop2 pny pnf* quadruple mutant; no meristem arrest. (D) *tga1 pny pnf* triple mutant; no meristem arrest. (E) *tga4 pny pnf* triple mutant; no meristem arrest. (F) *tga1 tga4 pny pnf* quadruple mutant; no meristem arrest. Scale bars, 0.5 cm.

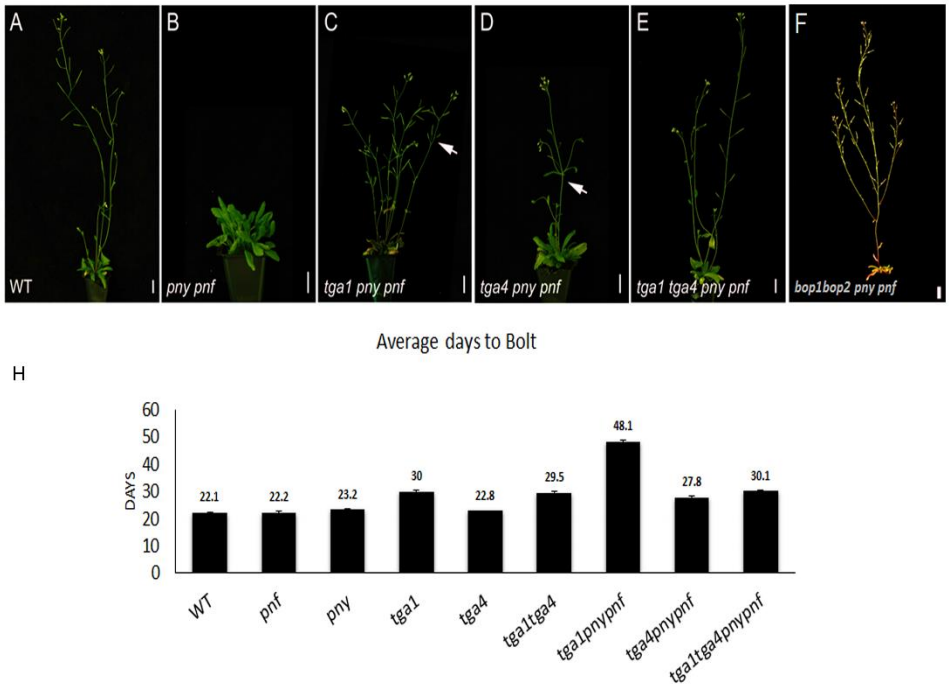
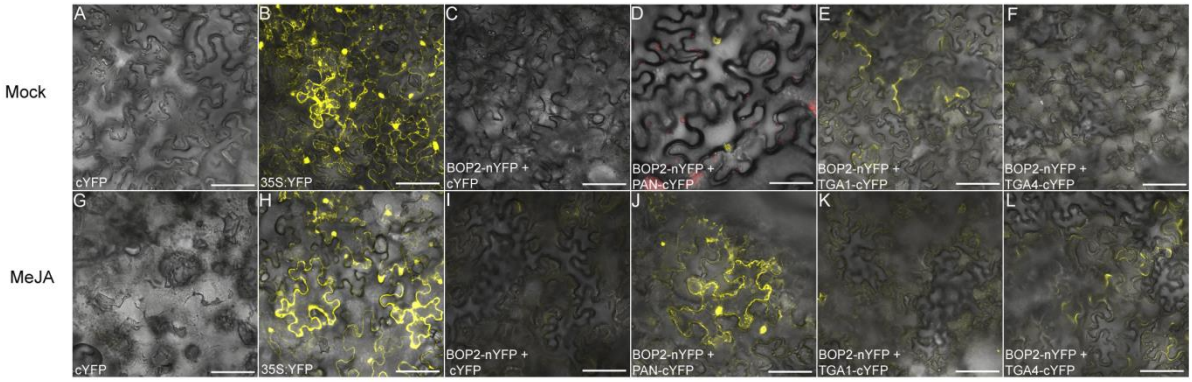


Figure 3.9 Inactivation of *TGA1* and *TGA4* rescues internode and flower formation in *pny pnf* mutants

(A-F) Representative 6 to 8-week-old plants are shown. (A) Wild-type control. (B) *pny pnf* mutant; non-flowering. (C) *tga1 pny pnf* mutant showing partial rescue of internode elongation and flowering; *pny*-like clustering of fruits (arrow). (D) *tga4 pny pnf* mutant showing partial rescue of internode elongation and flowering; *pny*-like clustering of fruits (arrow). (E) *tga1 tga4 pny pnf* mutant showing complete rescue of internode elongation and flowering; similar to wild-type. (F) *bop1 bop2 pny pnf* mutant also showing complete rescue. Scale bars, 1.5 cm. (H) Quantitative analysis of flowering time in wild-type and mutants. Average bolting date under continuous light was measured for at least twelve plants per genotype. Error bars, S.E.M.

Figure 3.10 Bimolecular fluorescence complementation fails to detect an interaction between BOP2 and Clade I TGA factors

The BiFC technique was used to investigate BOP2 interactions with clade I TGA factors *in planta*. The N-termini of YFP (nYFP) or C-termini of YFP (cYFP) were cloned in-frame to make fusions at the C-terminus of BOP2 or TGA factors (PAN, TGA1, or TGA4). Constructs were transiently expressed in tobacco leaves 2-days after treatment with 100 µm MeJA or a Mock solution. Pairs of constructs were transiently expressed in tobacco leaves (Materials and Methods). Reconstitution of YFP fluorescence was examined by confocal microscopy. Adaxial surface of leaves was imaged. (A and G) cYFP alone as a negative control. No fluorescence. (B and H) *35S:YFP* as a positive control. Yellow fluorescence was detected in the cytoplasm and nucleus as expected. (C and I) BOP2-nYFP + cYFP. No fluorescence. (D and J) BOP2-nYFP +PAN-cYFP as a positive control. Yellow fluorescence was observed in the nucleus as expected and in the cytoplasm of MeJA-treated plants. (E and K) BOP2-nYFP + TGA1-cYFP. No fluorescence. (G-H) BOP2-nYFP + TGA4-cYFP. No fluorescence. Scale bar, 100 µm. Images kindly provided by Keith Hubbard/Denise Chabot at Agriculture Canada.


Mock
MeJA
A
cYFP
B
35S:YFP
C
BOP2-nYFP +
cYFP
D
BOP2-nYFP +
PAN-cYFP
E
BOP2-nYFP +
TGA1-cYFP
F
BOP2-nYFP +
TGA4-cYFP
G
cYFP
H
35S:YFP
I
BOP2-nYFP +
cYFP
J
BOP2-nYFP +
PAN-cYFP
K
BOP2-nYFP +
TGA1-cYFP
L
BOP2-nYFP +
TGA4-cYFP

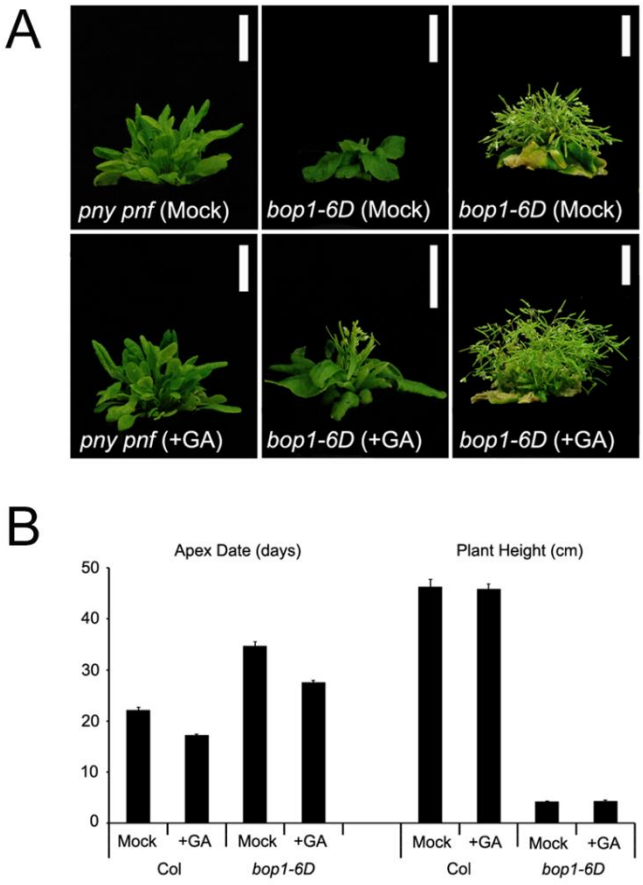
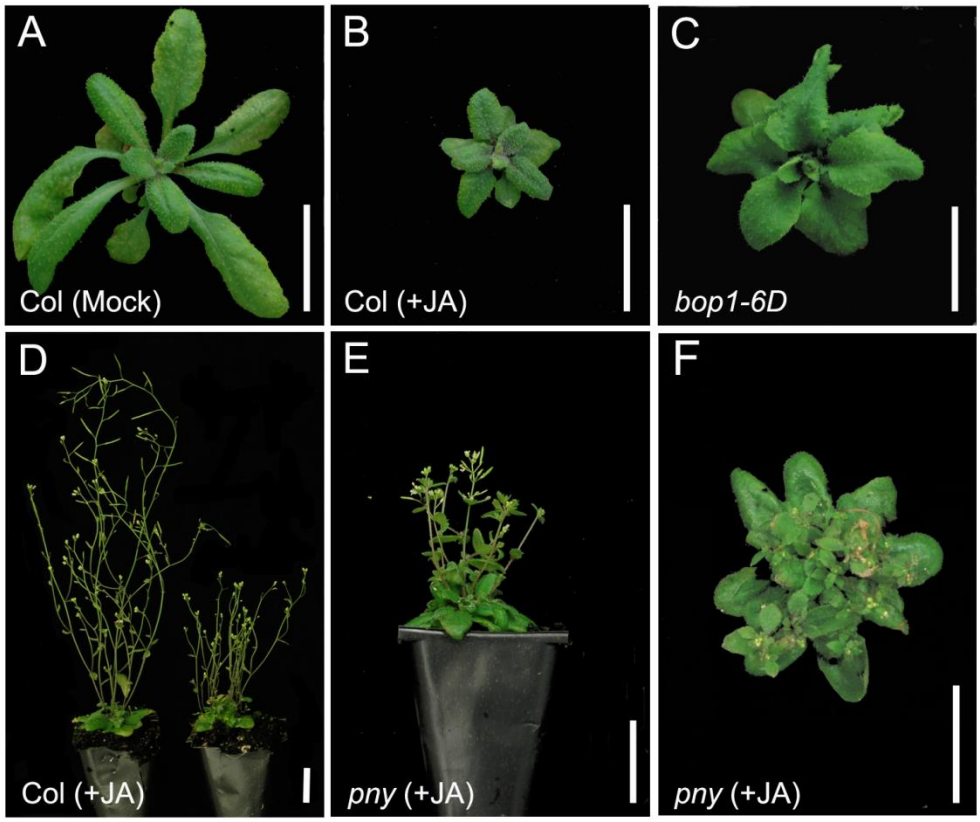


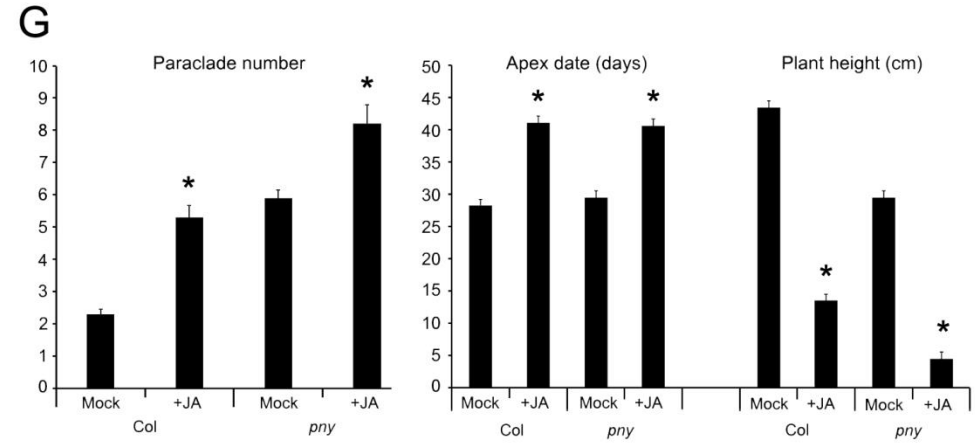
Figure 3.11. Exogenous GA_3 fails to rescue *pny pnf* or *bop1-6D* defects in internode elongation and flowering

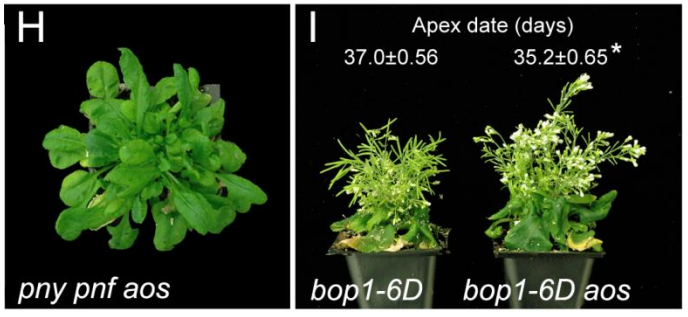
(A) *pny pnf* and *bop1-6D* plants treated with 100 µm GA_3 or a Mock solution. (B) Flowering time and plant height of Col and *bop1-6D* plants treated with 100 µm GA_3 or a Mock solution. Scale bars, 1.5 cm. Error bars represent, S.E.M (n=24)

Figure 3.12 Loss or gain of JA content effect on phenotype of wild-type and mutants

Wild-type and *pny* plants grown under LDs were sprayed daily until maturity with 100 µm MeJA or a Mock solution. (A) Mock-treated Col plant. (B) MeJA-treated Col plant showing small, dark green leaves. (C) Gain-of-function *bop1-6D* mutant showing a compact rosette similar to (B). (D) MeJA-treated Col plants showing *pny*-like partial loss of apical dominance and short stature. (E) MeJA-treated *pny* mutant showing enhancement of defects in internode elongation and apical dominance relative to Mock control (see G). (F) MeJA-treated *pny* mutant showing delayed flowering relative to Mock control. (G) Quantitative phenotypic analysis of wild-type and *pny* mutant plants treated with MeJA. For both genotypes, treatment with MeJA resulted in additional rosette paraclades indicating loss of apical dominance, reduced height, and delayed flowering. Asterisks indicate significant differences (Student's *t* test, $p<0.05$). Effect of *aos* loss-of-function on *pny pnf* and *bop1-6D* phenotypes. Representative plants are shown. H, *pny pnf aos* mutant remains non-flowering. (I-J) Phenotype of *bop1-6D* versus *bop1-6D aos* mutants. A small but highly significant ($p<0.0001$) increase in plant height (+1.26 cm) and earlier flowering (-1.8 days) is measured in *bop1-6D aos* compared to *bop1-6D* control plants. Analysis was performed in a *bop1-6D/+ aos/+* segregating population (n=100). Scale bars, 1.5 cm.


A
Col (Mock)
B
Col (+JA)
C
bop1-6D
D
Col (+JA)
E
pny (+JA)
F
pny (+JA)


G
Paraclade number
Apex date (days)
Plant height (cm)
Mock
+JA
Col
pny


H
pny pnf aos
I
Apex date (days)
37.0±0.56
35.2±0.65*
bop1-6D
bop1-6D aos
J
Plant height (cm)
bop1-6D
bop1-6D aos

CHAPTER 4

DISCUSSION

Plant development relies on the activity of the SAM which provides daughter cells for incorporation into aerial organs throughout the life cycle. In response to floral inductive signals, the SAM undergoes restructuring to become an IM resulting in new patterns of aerial development and the production of an inflorescence. TALE homeodomain proteins that maintain stem-cell identity facilitate this transition. Mutations in PNY and PNF support meristem production of leaves, but internode elongation and floral initiation are blocked (Smith et al., 2004). This failure is caused by misexpression of lateral organ boundary genes in *pny pnf* apices including *BOP1/2* and *ATH1/KNAT6* (Tabb, 2012). My thesis work addresses the mechanism.

My data provide evidence that BOP1/2 activates *ATH1* through a 2-kb fragment of promoter directly upstream of the ATG start site. This sequence contains multiple close matches to the consensus binding site for TGA bZIP transcription factors which are required in recruitment of BTB-ankryin factors to DNA. In support of this model, I provide evidence that clade I TGA1 and TGA4 factors expressed at lateral organ boundaries function in the same genetic pathway as BOP1/2 and ATH1/KNAT6. Lastly, my analysis of GA and JA hormone response provide evidence that genes in this module blocks meristem responsiveness to floral inductive signals at least in part through promotion of JA.

4.1 Characterization of loss and gain-of-function *ATH1* alleles

BOP1/2 are proposed to block flowering and internode elongation in *pny pnf* mutants *via* direct transcriptional promotion of *ATH1*. Consistent with this model, an *ath1-3* mutation fully rescues *pny pnf* defects (Tabb, 2012) but *ath1-1* alleles (Rutjens, 2009 #209) and

ath1-4 alleles (this study) do not. My analysis determined that *ath1-3* is a null allele in contrast to *ath1-1* and *ath1-4* alleles that express full or partial mutant transcripts. TALE homeodomain factors have three conserved functional domains: a SKY domain and adjacent BELL domain that mediate protein-protein interactions and homeodomain for DNA-binding at the C-terminus (Bellaoui et al., 2001; Fig. 3.1). The *ath1-1* mutant transcript is predicted to encode a partial protein that contains the SKY domain and part of the BELL domain. The *ath1-4* mutant transcript is predicted to encode a protein that terminates midway through the homeodomain due to a premature stop codon (Li et al., 2012). The truncated proteins produced by these alleles may retain partial function and bind to other TALE members including STM to form non-functional complexes in causing a dominant negative effect.

Plants that overexpress *BOP1/2* and *KNAT6* are restricted in growth resulting in short bushy inflorescences with clusters of fruits on the primary and secondary stems. My data confirm that ATH1 gain-of-function has a similar effect. Presumably, BOP1/2 and its downstream effectors ATH1/KNAT6 activate similar targets when overexpressed. Overexpression of a miR164-resistant version of the *CUC2* lateral organ boundary genes has a similar phenotype (Peaucelle et al., 2007). This suggests that BOP1/2-ATH1-KNAT6 and CUC members have overlapping and/or similar targets in restricting growth at lateral organ boundaries.

4.2 Clade I bZIP TGA factors are potential BOP co-factors

A steroid induction system was used to define a 2-kb fragment of the *ATH1* promoter that is responsive to BOP1 induction after 4 hours of DEX treatment. These data are in line with previous results showing induction of *ATH1* transcript after 2 and 4 hours of DEX treatment (Khan, 2013; Khan et al., 2015). ChIP assays showed enrichment of BOP1-GR protein at two positions in the *ATH1* promoter containing A-box motifs which function as TGA binding sites (Schindler et al., 1992; Izawa et al., 1993). These data support the model that TGA bZIP proteins are cofactors for BOP recruitment to the *ATH1* promoter.

Arabidopsis TGA factors are organized into five clades whose members have overlapping functions in development and defense against biotic and abiotic stress (Jakoby et al., 2002; Gatz, 2013). Previous experiments showed that Clade V factor PERIANTHIA (PAN/TGA8) functions in a complex with BOP1/2 to promote floral meristem identity and to control sepal/petal number in flowers (Hepworth et al., 2005; Xu et al., 2010). I show here that clade I factors TGA1 and TGA4 function in the same genetic pathway as BOP1/2 and ATH1/KNAT6 in regulation of meristem maintenance and flowering. Joint inactivation of *TGA1* and *TGA4* fully rescues *pny* and *pny pnf* defects in inflorescence architecture and flowering whereas single inactivation of *TGA1* or *TGA4* does not. Single inactivation of *TGA1* or *TGA4* is sufficient to rescue the SAM arrest phenotype of *pny pnf* mutants. However, onset of flowering in *tga1 pny pnf* triple mutants was significantly delayed in comparison to *tga4 pny pnf* mutants. Fruit clustering in *tga1 pny* mutants was rescued to a greater extent than in *tga4 pny* mutants. This pattern may reflect differences in *TGA1* and *TGA4* spatial patterns of expression in the stem. *TGA1p:GUS* and *TGA4p:GUS* reporter

genes have a similar pattern of expression in the vasculature of young leaves and at lateral organ boundaries in the inflorescence, but their expression differs in vascular tissues of the stem (Devi, 2014). *TGA1* is expressed in xylem elements whereas *TGA4* is additionally expressed in phloem which is important for transport of FT, GAs, and sugar to the shoot apex (Srikanth and Schmid, 2011; Proveniers, 2013; Wang, 2014). Future experiments will determine if these *TGA1* and *TGA4* are misexpressed in *pny pnf* apices as shown for other module members (Tabb, 2012; Khan et al., 2015). In addition, ChIP assays will be used to test for co-localization of TGA1 and/or TGA4 and BOP1 at A-box sites in the *ATH1* promoter.

4.3 Visualizing BOP-TGA interactions *in vivo*

Despite multiple lines of evidence supporting that BOP1/2 and TGA1/4 function in the same genetic pathway (Khan, 2013; Devi, 2014; this study) no protein-protein interaction was detected using BiFC in tobacco leaves, even when sprayed with 100 μm MeJA to stimulate stress conditions. In contrast, an interaction between BOP2 and PAN was readily detected. Previous attempts to detect an interaction between BOP1/2 and TGA1/4 have also failed (Hepworth et al., 2005; Devi, 2014). Biochemical studies have showed that enzymatic reduction of Cys260 and Cys266 residues in TGA1 (and the equivalent residues in TGA4) is required for interaction with NPR1 and activation of defense genes (Després et al., 2003). Site directed mutants of TGA1 that mimic a reduced state (Cys260Asp Cys266Ser) enable constitutive interactions with NPR1 in yeast and *in planta* (Després et al., 2003). S-nitrosylation or S-glutathionylation of these residues have the same effect

(Lindermayr et al., 2010). Future work will test if similar regulatory modifications of TGA1 and TGA4 are required for interaction with BOP1/2 in plant development.

4.4 JA-GA antagonistic regulation of flowering and internode elongation

My thesis work tested the hypothesis that lateral organ boundary genes in the BOP module interfere with internode elongation and flowering by perturbing GA and JA hormone pathways. A large body of work shows that GAs function as endogenous growth regulators that promote bolting and flower formation when other pathways are inactive (Mutasa-Göttgens and Hedden, 2009). In short days, the GA pathway is obligatory for the activation of floral integrator genes *SOC1*, *LFY*, and *FT* in promotion of flowering. In long days, GA contributes to flowering via the miR156-SPL-miR172 module (Galvão et al., 2012; Porri et al., 2012). The transcript profile of *pny pnf* apices and *bop1-6D* mutants suggests blockage at multiple nodes of the GA pathway including biosynthesis, catabolism, and signaling (Khan et al., 2015). Consistent with this, exogenous GA treatment was insufficient to rescue *pny pnf* non-flowering or stem elongation defects in *bop1-6D* mutants although flowering was 3-4 days earlier in this mutant.

Microarray profiling of *BOP1* overexpressing lines prompted us to form the hypothesis that activation of stress response pathways leads to accumulation JA as a factor in repression of GA signaling and IM activity. Studies in *Nicotiana attenuata* showed that high levels of JA antagonize the biosynthesis of GAs and inhibit the growth of stems (Heinrich et al., 2012). MeJA is also shown to have an inhibitory effect on flowering and stem elongation in *Pharbitis nil* (Maciejewska and Kopceiwicz, 2002). An effect for JA on

flowering-time is not well characterized in Arabidopsis. I show here that exogenous treatment of wild-type plants with MeJA stunts the growth of leaves, impairs apical dominance, delays flowering, and reduces stem elongation. All of these phenotypes are characteristics of *pny* and *pny pnf/+* mutants. Thus, treatment of wild-type plants with MeJA induces the same spectrum of defects as seen in *pny* and *pny pnf/+* mutants. Inhibitory effects of MeJA on flowering are reported for a few species including wheat (Albrechtová and Ullmann, 1994; Maciejewska and Kopceiwicz, 2002; Maciejewska et al., 2004; Diallo et al., 2014). In some cases, these phenotypes are linked to repression of GA biosynthesis (Magome et al., 2004; Magome et al., 2008; Heinrich et al., 2012), stabilization of DELLAs (Yang et al., 2012) and/or induction of AP2/ERF factors (Magome et al., 2008; Sun et al., 2008; Kang et al., 2011; Licausi et al., 2013).

4.5 Inactivation of JA biosynthetic gene *AOS* fails to rescue flowering in *pny pnf* but modifies the *bop1-6D* phenotype

Allene oxide synthase which catalyzes dehydration of hydroperoxide to allene oxide is one of the first steps in biosynthesis of JA (Park et al., 2002). Endogenous JA levels which increase 100-fold after wounding in wild-type plants do not increase in this mutant (Park et al., 2002). Introgression of the *aos* mutation into *pny*, *pny pnf*, and *bop1-6D* mutants was used to test if a reduction in JA content suppresses defects in SAM maintenance, internode elongation, or flowering. No rescue of *pny* (data not shown) or *pny pnf* defects was observed but there was a small but significant increase in plant height and flowering time for *bop1-6D aos* mutants. This suggests that JA is one of several factors that play a role in blocking IM activity in *pny pnf* and *bop1-6D* mutants. FD levels required for FT

function are also reduced in *pny pnf* apices and may contribute to the phenotype (Khan et al., 2015).

4.6 Model for integration of JA stress signals *via* the miR156-SPL-miR172 module

Deficiency in miR156-regulated *SPL* transcript abundance in *pny pnf* apices was previously shown to have biological relevance (Lal et al., 2011; Khan et al., 2015). Transgenic *pny pnf* plants expressing a miR156-resistant form of *SPL4* were restored for *LFY* and *AP1* expression but only partly restored for flowering suggesting that flowering rescue may require the concerted activity of multiple *SPL* factors (Lal et al., 2011). We currently favor a model in which promotion of stress signals including JA by BOP1/2 repress flowering by modulating components of the miR156-SPL-miR172 module. Recent data provide compelling evidence that stress signals are integrated through this module. Cui et al. (2014) showed that delayed flowering in response to stress stimuli correlates with increased abundance of miR156 leading to down-regulation of *SPL9*. Compatible with this, plants overexpressing miR156 show increased tolerance to stress and are very late flowering. Stief et al. (2014) similarly showed that induction of miR156a-f isoforms by heat stress correlates with down-regulation of *SPL9*-like transcripts (*SPL2*, *SPL9*, *SPL11*) and delayed flowering. My study is the first evidence that lateral organ boundary genes modulate growth and meristem activity *via* JA biosynthesis.

4.7 Concluding remarks

In summary, my data help to explain how genes in the BOP module repress meristem activity and flowering in *pny pnf* mutants. I found the location of elements in a region of the *ATH1* promoter that shows responsiveness to BOP1 induction and provided genetic data to show that clade I TGA factors are part of this pathway. My data also provide evidence that BOP1/2 promotion of JA is one of several factors that contributing to inhibition of growth and IM activity in *pny pnf* mutants. These data provide new insight into mechanisms for integration of stress signals in control of flowering and uncover a previously undiscovered potential role for jasmonate in regulation of growth and meristem activity at lateral organ boundaries.

CHAPTER 5

GENERAL CONCLUSION AND FUTURE DIRECTIONS

5.1 General conclusion

BOP1/2 and ATH1/KNAT6 are lateral organ boundary genes whose interacting products form a module that regulates growth, meristem function, and patterning at junctions in the plant. This module is a potent antagonistic of IM activity essential for flowering.

My thesis contributed in three areas. First, my study of regulatory interactions between BOP1/2 and ATH1 components confirmed the functions of the factors in the same genetic pathway. Second, I showed that clade I TGA bZIP factors TGA1 and TGA4 are components of this module and likely involved in recruitment of BOP1/2 to the *ATH1* promoter. BOP1/2 interaction with TGA1/4 may help explain a dual role for both of these factors in development and defense (Khan et al., 2014). Finally, I used BOP1 overexpression lines as a tool to understand the basis of non-flowering in *pny pnf* mutants. These studies provide preliminary evidence that promotion of jasmonic acid signaling by lateral organ boundary genes is a factor in inhibiting meristem responsiveness to floral inductive signaling. These data have important implications for understanding cross-talk between stress pathways and flowering. My data also shed new light on a role for lateral organ boundary genes in production of JA for regulation of axillary meristem initiation, abscission, dehiscence and plant defense.

5.2 Future directions

5.2.1 Class I bZIP factors as BOP-interacting co-factors in regulation of gene expression

My data are consistent with the model that BOP1/2 bind *via* clade I TGA factors to A-box sites in the *ATH1* promoter to activate transcription. However, biochemical evidence for this model is lacking.

5.2.1.1 Optimization of BiFC experiments

Future work will focus on optimizing the BiFC technique to test this model. Several factors may be at play. The geometry of the interaction may fail to bring the two halves of YFP close enough together to restore fluorescence in the case of TGA1 and TGA4. Fusing YFP to the N-terminus of BOP1/2 and TGA1/4 may be required. Alternatively, the interaction may require a co-factor or post-translational modification of BOP1/2 or TGA residues.

Studies with NPR1 show that salicylic acid (SA) binds to Cys residues in a C-terminal regulatory domain to trigger a conformational change required for activation of defense genes (Wu et al., 2012). Enzymatic reduction of Cys260 and Cys266 residues in TGA1 (and the equivalent residues in TGA4) also takes place in the presence of SA and is required for an interaction with NPR1 (Després et al., 2003). Oxidizing factors such a nitric oxide have a similar effect (Lindermayr et al., 2010). Mutants forms of TGA1 with Cys260/Cys266 are replaced with Asp/Ser (or equivalent residues in TGA4) interact constitutively with NPR1

(Després et al., 2003). These same conditions will be tested for application to BOP1/2-TGA1/4 interactions.

Experiments with MeJA will also continue. Several lines of evidence including microarray data, transcript profiling, and phenotypic studies strongly support that BOP1/2 activity interacts with JA signaling pathways (Canet et al., 2012; Khan et al., 2015; this study). There are many reasons why spray treatment of tobacco leaves with 100 µm MeJA had no stimulating effect on an interaction between BOP2 and TGA1/4. For one, it is possible that insufficient hormone was delivered to leaf cells. Indeed, we were unable to detect any interaction between NPR1 with TGA1/4 when tobacco leaves were sprayed with 1 mM SA. The leaves become necrotic (yellow) and died. Future experiments will focus on establishing reliable conditions for detecting NPR1-TGA1/4 control interactions. Once this is established, similar conditions will be used for monitor of BOP1/2 and TGA1/4 interactions using a variety of stimuli including SA, ethylene, MeJA, and oxidizing agents such as hydrogen peroxide or nitric oxide.

If consistent delivery of these agents is difficult in tobacco leaves, we can switch to BiFC carried out in Arabidopsis leaf protoplasts or Arabidopsis cotyledons where we can use greater precision to control hormone dosage (Marion et al., 2008; Lindermayr et al., 2010; Xu et al., 2010).

5.2.1.2 ChIP experiments

Our study identified two A-box sites in the *ATH1* promoter that show enrichment of BOP1-GR protein *in vivo* using ChIP assays. ChIP experiments will take place to test if TGA1 or TGA4 co-localize to these same sites. Transgenic lines will be created containing TGA1-GFP and TGA4-GFP fusion proteins expressed in wild-type and *tga1 tga4* genetic backgrounds under the control of an endogenous promoter or a strong constitutive viral 35S CaMV promoter. ChIP assays will be carried out as described in Khan et al. (2015) using an anti-GFP antibody for co-immunoprecipitation of protein-DNA complexes. In parallel, the *tga1 tga4* mutation will be introgressed into 2.0-kb *ATH1pGUS 35S:BOP1-GR* promoter line by crossing. This will allow us to test if BOP1 induction of ATH1 is dependent on TGA1/4 activity. This same line can also be used to test if TGA1 and TGA4 activity is required for BOP1-dependent induction *ATH1* transcript by qRT-PCR.

5.2.2 Integration of stress and sugar signals by the miR156-SPL-miR172 module

My data provide evidence for JA as a potential factor in modulating *bop1-6D* and *pny pnf* phenotypic defects. However, inactivation of an *AOS* biosynthetic enzyme failed to rescue flowering in *pny pnf* showing that additional factors are involved. We hope to identity these factors.

5.2.2.1 Stress signals

Although flowering does not occur in *aos pny pnf* mutants it might be useful to test if accumulation of floral-meristem transcripts (*FT/FD*, *LFY*, *SPLs*, and *AP1*) is partially restored. There is also the possibility that *aos* mutations are not effective in blocking JA signaling as this has never been tested. As a control, it might be useful to determine if flowering is rescued by a mutation in the CORONATINE INSENSITIVE1 receptor for JA required in defense signaling (Xie et al., 1998; Yan et al., 2009). Ontology (GO) analysis of differentially regulated genes revealed broad enrichment of terms associated with response to biotic and abiotic stress (Appendix I). Whilst response to jasmonic acid (JA) stimulus (GO:009753) was at the top of the list, other hormone pathways associated with stress showed similar enrichment. In descending order these were response to salicylic acid stimulus (GO:0009751), response to ethylene stimulus (GO:009723) and response to abscisic acid stimulus (GO:0099737). Thus, it may be not be possible to rescue flowering in a *pny pnf* mutant by targeting JA alone.

5.2.2.2 Sugar

The switch to reproductive development requires mobile carbohydrate to fuel an increased rate of cell division in aerial tissues for production of inflorescences, flowers, and seeds. Plants measure sugar status in leaves and in the shoot apex by monitoring the abundance of trehalose-6-phosphate (T6P) (Matsoukas et al., 2012; Wahl et al., 2013). Production of T6P is upregulated in shoot apices during the transition to flowering which in turn promotes SPL3/4/5 expression in the SAM (Wingler et al., 2012; Wahl et al., 2013).

GO enrichment analysis of differentially expressed genes in the *bop1-6D* mutant is suggestive of down-regulation of cellular carbohydrate metabolism, metabolic processes, and nitrogen metabolism, which potentially restrict sucrose availability at the shoot apex (Appendix I). This fits with the model that metabolic resources in *bop1-6D* are redirected towards defense against biotic and abiotic stresses. Given that TPS1 loss-of-function delays or completely block flowering (Wahl et al., 2013) this connection is worth investigating. For example, does inactivation of *aos* combined with the addition of exogenous sugar and GA rescue flowering? Answers to these questions await further investigation.

Collectively, these studies will pave the way to a greater understanding the developmental mechanisms governing stress signals and the transition to flowering in plants.

REFERENCES

Abe M, Kobayashi Y, Yamamoto S, Daimon Y, Yamaguchi A, Ikeda Y, Ichinoki H, Notaguchi M, Goto K, Araki T (2005) FD, a bZIP protein mediating signals from the floral pathway integrator FT at the shoot apex. Science **309:** 1052-1056

Aida M, Ishida T, Fukaki H, Fujisawa H, Tasaka M (1997) Genes involved in organ separation in Arabidopsis: an analysis of the *cup-shaped cotyledon* mutant. Plant Cell **9:** 841-857

Aida M, Ishida T, Tasaka M (1999) Shoot apical meristem and cotyledon formation during Arabidopsis embryogenesis: interaction among the CUP-SHAPED COTYLEDON and SHOOT MERISTEMLESS genes. Development **126:** 1563-1570

Aida M, Tasaka M (2006a) Genetic control of shoot organ boundaries. Curr Opin Plant Biol **9:** 72-77

Aida M, Tasaka M (2006b) Morphogenesis and patterning at the organ boundaries in the higher plant shoot apex. Plant Mol Biol **60:** 915-928

Albrechtová J, Ullmann J (1994) Methyl jasmonate inhibits growth and flowering in *Chenopodium rubrum*. Biol Plantarum **36:** 317-319

Amasino RM, Michaels SD (2010) The timing of flowering. Plant Physiol **154:** 516-520

Andrés F, Coupland G (2012) The genetic basis of flowering responses to seasonal cues. Nat Rev Genet **13:** 627-639

Axtell M, Bowman JL (2009) Evolution of plant microRNAs and their targets. Trends Plant Sci **13:** 343-349

Barton K (2010) Twenty years on: the inner workings of a shoot apical meristem, a developmental dynamo. Dev Biol **341:** 95-113

Barton MK, Poethig RS (1993) Formation of the shoot apical meristem in *Arabidopsis thaliana*: an analysis of development in the wild-type and in the *shoot meristemless* mutant. Development **119:** 823-831

Bellaoui M, Pidkowich MS, Samach A, Kushalappa K, Kohalmi SE, Modrusan Z, Crosby WL, Haughn WG (2001) The Arabidopsis BELL1 and KNOX TALE homeodomain proteins interact through a domain conserved between plants and animals. Plant Cell **13:** 2455-2470

Belles-Boix E, Hamant O, Witiak SM, Morin H, Traas J, Pautot V (2006) *KNAT6*: an Arabidopsis homeobox gene involved in meristem activity and organ separation. Plant Cell **18:** 1900-1907

Bergonzi S, Albani MC (2011) Reproductive competence from an annual and a perennial perspective. J Exp Bot **62:** 4415-4422

Bernier G (1988) The control of floral evocation and morphogenesis. Annu Rev Plant Physiol **39:** 175-219

Bevan M, Walsh S (2005) The Arabidopsis genome: a foundation for plant research. Genome Res **15:** 1632-1642

Bonaventure G, Gfeller A, Proebsting WM, Hörsteiner S, Chételat A, Martinoia E, Farmer EE (2007) A gain-of-function allele of TPC1 activates oxylipin biogenesis after leaf wounding in Arabidopsis. Plant J **49:** 899-898

Bowman J, Alvarez J, Weigel D, Meyerowitz E (1993) Control of flower development in *Arabidopsis thaliana* by *APETALA1* and interacting genes. Development **119:** 721-743.

Bowman JL, Eshed Y (2000) Formation and maintenance of the shoot apical meristem. Trends Plant Sci **5:** 110-115

Boyle P, Le Su E, Rochon A, Shearer HL, Murmu J, Chu JY, Fobert PR, Despres C (2009) The BTB/POZ domain of the Arabidopsis disease resistance protein NPR1 interactis with the repression domain of TGA2 to negate its function. Plant Cell **21:** 3700-3713

Brand U, Fletcher J, Hobe M, Meyerowitz EM, Simon R (2000) Dependence of stem cell fate on Arabidopsis on a feedback loop regulated by CLV3 activity. Science **289:** 617-619

Bryne ME, Simorowski J, Martienssen RA (2002) ASYMMETRIC LEAVES1 reveals KNOX gene redundancy in Arabidopsis. Development **129:** 1957-1965

Byrne ME, Groover AT, Fontana JR, Martienssen RA (2003) Phyllotactic pattern and stem cell fate are determined by the Arabidopsis homeobox gene *BELLRINGER*. Development **130:** 3941-3950

Canet J, Dobón A, Fajmonová J, Tornero P (2012) The *BLADE-ON-PETIOLE* genes of Arabidopsis are essential for resistance induced by methyl jasmonate. BMC Plant Biol **12:** 199

Capron A, Chatfield S, Provart N, Berleth T (2009) Embryogenesis: pattern formation from a single cell. The Arabidopsis Book **e0126:** 10.1199/tab.0126

Chakravarthy S, Tuori RP, D'Ascenzo MD, Fobert PR, Després C, Martin GB (2003) The tomato transcription factor Pti4 regulates defense-related gene expression via GCC box and non-GCC box cis elements. Plant Cell **15:** 3033-3050

Cipollini D (2005) Interactive effects of lateral shading and jasmonic acid on morphology, phenology, seed production,and defense traits in *Arabidopsis thaliana*. Int J Plant Sci **166:** 955-959

Clark SE, Jacobsen SE, Levin JZ, Meyerowitz EM (1996) The *CLAVATA* and *SHOOT MERISTEMLESS* loci competitively regulate meristem activity in Arabidopsis. Development **122:** 1567-1575

Clough SJ, Bent AF (1998) Floral dip: a simplified method for Agrobacterium-mediated transformation of *Arabidopsis thaliana*. Plant J **16:** 735-743

Corbesier L, Vincent C, Jang S, Fornara F, Fan Q, Searle I, Giakountis A, Farrona S, Gissot L, Turnbull C, Coupland G (2007) FT protein movement contributes to long-distance signaling in floral induction of Arabidopsis. Science **316:** 1030-1033

Cui L-G, Shan J-X, Shi M, Gao J-P, Lin H-X (2014) The miR156-SPL9-DFR pathway coordinates the relationship between development and abiotic stress tolerance in plants. Plant J **80:** 1108-1117

Després C, Chubak C, Rochon A, Clark R, Bethune T, Desveaux D, Fobert PR (2003) The Arabidopsis NPR1 disease resistance protein is a novel cofactor that confers redox regulation of DNA binding activity to the basic domain/leucine zipper transcription factor TGA1. Plant Cell **15:** 2181-2191

Després C, DeLong C, Glaze S, Liu E, Fobert PR (2000) The Arabidopsis NPR1/NIM1 protein enhances the DNA binding activity of a subgroup of the TGA family of bZIP transcription factors. Plant Cell **12:** 279-290

Devi B (2014) Investigating a conserved role for BLADE-ON-PETIOLE and class I TGA bZIP transcription factors in regulation of inflorescence architecture and lignin biosynthesis in *Arabidopsis thaliana* and *Populus trichocarpa*. Carleton University, Ottawa

Diallo A, Agharbaoui Z, Badawi M, Ali-Benali M, Moheb A, Houde M, Sarhan F (2014) Transcriptome analysis of an *mvp* mutant reveals important changes in global gene expression and a role for methyl jasmonate in vernalization and flowering in wheat. J Exp Bot **65:** 2271-2286

Dill A, SG T, J H, CM S, Sun T-P (2004) The Arabidopsis F-box protein SLEEPY1 targets gibberellin signaling repressors for gibberellin-induced degradation. Plant Cell **16:** 1392-1405

Edwards K, Johnstone C, Thompson C (1991) A simple and rapid method for the preparation of plant genomic DNA for PCR analysis. Nucleic Acid Res **19:** 1349

Ellis C, I. K, Wasternak C, Turner JG (2002) The Arabidopsis mutant *cev1* links cell wall signaling to jasmonate and ethylene responses. Plant Cell **14:** 1557-1566

Endrizzi K, Moussian B, Haecker A, Levin JZ, Laux T (1996) The *SHOOT MERISTEMLESS* gene is required for maintenance of undifferentiated cells in the Arabidopsis shoot and floral meristems and acts at a different regulatory level than the meristem genes *WUSCHEL* and *ZWILLE*. Plant J **10:** 967-979

Eriksson S, Böhlenius H, Moritz T, Nilsson O (2006) GA_4 is the active gibberellin in the regulation of *LEAFY* transcription and Arabidopsis floral initiation Plant Cell **18:** 2171-2181

Ferrier T, Matus JT, Jin J, Reichmann JL (2011) Arabidopsis paves the way: genomic and network analyses in crops. Curr Opin Biotech **22:** 260-270

Fletcher J (2002) Shoot and floral meristem maintenance in Arabidopsis. Annu Rev Plant Biol **53:** 45-66

Fletcher J, Brand U, Running MP, Simon R, Meyerowitz EM (1999) Signaling of cell fate decisions by CLAVATA3 in Arabidopsis shoot meristems. Science **283:** 1911-1914

Fode B, Siemsen T, Thurow C, Weigel R, Gatz C (2008) The Arabidopsis GRAS protein SCL14 interacts with class II TGA transcription factors and is essential for the activation of stress-inducible promoters. Plant Cell **20:** 3122-3135

Gallois J-L, Woodward C, Reddy GV, Sablowski R (2002) Combined SHOOT MERISTEMLESS and WUSCHEL triger ectopic organogenesis in Arabidopsis. Development **129:** 3207-3217

Galvão V, Horrer D, Küttner F, Schmid M (2012) Spatial control of flowering by DELLA proteins in *Arabidopsis thaliana*. Development **139:** 4072-4082

Gatz C (2013) From pioneers to team players: TGA transcription factors provide a molecular link between different stress pathways. Mol Plant Microb Interact **26:** 151-159

Gómez-Mena C, Sablowski R (2008) *ARABIDOPSIS THALIANA HOMEOBOX GENE1* establishes the basal boundaries of shoot organs and controls stem growth. Plant Cell **20:** 2059-2072

Guo M, Thomas J, Collins G, Timmermans MCP (2008) Direct repression of KNOX loci by the ASYMMETRIC LEAVES1 complex of Arabidopsis. Plant Cell **20:** 48-53

Ha CM, Jun JH, Fletcher JC (2010) Control of Arabidopsis leaf morphogenesis through regulation of the YABBY and KNOX families of transcription factors. Genetics **186:** 197-206

Ha CM, Jun JH, Nam HG, Fletcher J (2004) BLADE-ON-PETIOLE1 encodes a BTB/POZ domain protein required for leaf morphogenesis in *Arabidopsis thaliana*. Plant Cell Physiol **45:** 1361-1370

Ha CM, Jun JH, Nam HG, Fletcher JC (2007) BLADE-ON-PETIOLE 1 and 2 control Arabidopsis lateral organ fate through regulation of LOB domain and adaxial-abaxial polarity genes. Plant Cell **19:** 1809-1825

Ha CM, Kim G-T, Kim BC, Jun JH, Soh MS, Ueno Y, Machida Y, Tsukaya H, Nam HG (2003) The BLADE-ON-PETIOLE1 gene controls leaf pattern formation through the modulation of meristematic activitiy in Arabidopsis. Development **130:** 161-172

Hamant O, Pautot V (2010) Plant development: a TALE story. CR Biol **333:** 371-381

Harberd NP, Belfield E, Yasumura Y (2009) The angiosperm gibberellin-GID1-DELLA growth regulatory mechanism: how an "inhibitor of an inhibitor" enables flexible response to fluctuating environments. Plant Cell **21:** 1328-1339

Hartmann U, Hohmann S, Nettesheim K, Wiseman E, Saedler H, Huijser P (2000) Molecular cloning of SVP: a negative regulator of the floral transition in Arabidopsis. Plant J **21:** 361-360

Hay A, Kaur H, Phillips A, Hedden P, Hake S, Tsiantis M (2002) The gibberellin pathway mediates KNOTTED1-type homeobox function in plants with different body plans. Curr Biol **12:** 1557-1565

Hay A, Tsiantis M (2010) KNOX genes: versatile regulators of plant development and diversity. Development **137:** 3153-3165

He Y, Amasino RM (2005) Role of chromatin modification in flowering-time control. Trends Plant Sci **10:** 30-35

Heinrich M, Hettenhausen C, Lange T, Wünsche H, Fang J, Baldwin I, Wu J (2012) High levels of jasmonic acid antagonize the biosynthesis of gibberellins and inhibit the growth of *Nicotiana attenuata* stems. Plant J **73:** 591-606

Hellens RP, Edwards EA, Leyland NR, Bean S, Mullineaux PM (2000) pGreen: a versatile and flexible binary Ti vector for Agrobacterium-mediated plant transformation. Plant Mol Biol **42:** 819-832

Helliwell CA, Wood CC, Robertson M, Peacock WJ, Dennis ES (2006) The Arabidopsis FLC protein interacts directly *in vivo* with SOC1 and FT chromatin and is part of high-molecular weight protein complex. Plant J **46:** 183-192

Henderson IR, Dean C (2004) Control of Arabidopsis flowering: the chill before the bloom. Development **131:** 3829-3838

Hepworth SR, Valverde F, Ravenscroft D, Mouradov A, Coupland G (2002) Antagonistic regulation of flowering-time gene *SOC1* by CONSTANS and FLC via separate promoter motifs. EMBO J **21:** 4327-4337

Hepworth SR, Zhang Y, McKim S, Li X, Haughn GW (2005) BLADE-ON-PETIOLE-dependent signaling controls leaf and floral patterning in Arabidopsis. Plant Cell **17:** 1434-1448

Huijser P, Schmid M (2011) The control of developmental phase transitions in plants. Development **138:** 4117-4129

Hyun Y, Choi S, Hwang H-J, J. Y, Nam S-J, Ko J, Park J-Y, Seo YS, Kim EY, Ryu SB, Kim WT, Lee YH, Kang H, Lee I (2008) Cooperation and functional diversification of two closely related galactolipase genes for jasmonate biosynthesis. Dev Cell **14:** 183-192

Izawa T, Foster R, Chua N-H (1993) Plant bZIP protein DNA binding specificity. J Mol Biol **230:** 1131-1144

Jaeger KE, Pullen N, Lamzin S, Morris RJ, Wigge PA (2013) Interlocking feedback loops govern the dynamic behavior of the floral transition in Arabidopsis. Plant Cell **25:** 820-833

Jaeger KE, Wigge PA (2007) FT protein acts as a long-range signal in Arabidopsis. Curr Biol **17:** 1050-1054

Jakoby M, Weisshaar B, Dröge-Laser W, Vincent-Carbajosa J, Tiedemann J, Kroj T, Parcy F (2002) bZIP transcription factors in Arabidopsis. Trends Plant Sci **7:** 106-111

Jefferson RA, Kavanagh TA, Bevan MW (1987) GUS fusions: ß-glucuronidase as a sensitive and versatile gene fusion marker in higher plants. EMBO J **6:** 3901-3907

Jun JH, Ha CM, Fletcher JC (2010) BLADE-ON-PETIOLE1 coordinates organ determinacy and axial polarity in arabidopsis by directly activating *ASYMMETRIC LEAVES2*. Plant Cell **22:** 62-76

Jung JH, Ju Y, Seo PJ, Lee JH, Park CM (2012) The SOC1-SPL module integrates photoperiod and gibberellic acid signals to control flowering time in Arabidopsis. Plant J **69:** 577-588

Kang H-G, Kim J, Kim B, Jeong H, Choi SH, Kim EK, Lee H-Y, Lim PO (2011) Overexpression of FTL1/DDF1, an AP2 transcription factor, enhances tolerance to cold, drought, and heat stresses in *Arabidopsis thaliana*. Plant Sci **180:** 634-641

Kanrar S, Bhattacharya M, Arthur B, Courtier J, Smith HMS (2008) Regulatory networks that function to specify flower meristems require the function of homeobox genes PENNYWISE and POUNDFOOLISH in Arabidopsis. Plant J **54:** 924-937

Kanrar S, Onguka O, Smith HM (2006) Arabidopsis inflorescence architecture requires the activities of KNOX-BELL homeodomain heterodimers. Planta **224:** 1163-1173

Kardailsky I, Shukla VK, Ahn JH, Dagenais N, Christensen SK, Nguyen JT, Chory J, Harrison MJ, Weigel D (1999) Activation tagging of the floral inducer FT. Science **286:** 1962-1965

Kesarwani M, Yoo J, Dong Y (2007) Genetic interactions of TGA transcription factors in the regulation of pathogenesis-related genes and disease resistance in Arabidopsis. Plant Physiol **144:** 336-346

Khan M (2013) Interactions of BLADE-ON-PETIOLE1and 2 with TALE homeobox genes in the regulation of flowering and inflorescence architecture in *Arabidopsis thaliana*. Carleton University, Ottawa

Khan M, Ragni L, Tabb P, Salasini BC, Chatfield S, Datla R, Lock J, Kuai X, Després C, Proveniers M, Yongguo C, Xiang D, Morin H, Citerne S, Hepworth SR, Pautot V (2015) Repression of lateral organ boundary genes by PENNYWISE and POUND-FOOLISH is essential for mersitem maintenance and flowering in *Arabidopsis thaliana*. Plant Physiol **submitted**

Khan M, Tabb P, Hepworth SR (2012a) BLADE-ON-PETIOLE1 and 2 regulate Arabidopsis inflorescence architecture in conjunction with homeobox genes KNAT6 and ATH1. Plant Signal Behav **7:** 788-792

Khan M, Xu H, Hepworth SR (2014) BLADE-ON-PETIOLE genes: setting boundaries in development and defense. Plant Sci **215-216:** 157-171

Khan M, Xu M, Murmu J, Tabb P, Liu Y, Storey K, McKim SM, Douglas CJ, Hepworth SR (2012b) Antagonistic interaction of BLADE-ON-PETIOLE1 and 2 with BREVIPEDICELLUS and PENNYWISE regulates Arabidopsis inflorescence architecture. Plant Physiol **158:** 946-960

Kim J, Lee JH, Kim W, Jung HS, Huijser P, Ahn JH (2012) The microRNA156-SQUAMOSA PROMOTER BINDING PROTEIN-LIKE3 module regulates ambient temperature-responsive flowering via FLOWERING LOCUS T in Arabidopsis. Plant Physiol **159:** 461-478

Kobayashi Y, Kaya H, Goto K, Iwabuchi M, Araki T (1999) A pair of related genes with antagonistic roles in mediating flowering signals. Science **286:** 1960-1962

Koncz C, Schell J (1986) The promoter of TL-DNA gene 5 controls the tissue-specific expression of chimeric genes carried by a novel type of Agrobacterium binary vector. Mol Gen Genet **204:** 383-396

Koornneef M, Meinke D (2010) The development of Arabidopsis as a model plant. Plant J **61:** 909-921

Lal S, Pacis LB, Smith HM (2011) Regulation of the SQUAMOSA PROMOTER-BINDING PROTEIN-LIKE genes/microRNA156 module by the homeodomain proteins PENNYWISE and POUND-FOOLISH in Arabidopsis. Mol Plant **4:** 1123-1132

Li D, Liu C, Shen L, Wu Y, Chen H, Robertson M, Helliwell CA, Meyerowitz EM, Hao Y (2008) A repressor complex governs the integration of flowering signals in Arabidopsis. Dev Cell **15:** 110-120

Li Y, Pi L, Huang H, Xu L (2012) ATH1 and KNAT2 proteins act together in regulation of plant inflorescence architecture. J Exp Bot **63:** 1423-1433

Licausi F, Ohme-Takagi M, Perata P (2013) APETALA2/Ethylene Responsive Factor (AP2/ERF) transcription factors: mediators of stress responses and developmental programs. New Phytol **199:** 639-649

Liljegren SJ, Gustafson-Brown C, Pinyopich A, Ditta GS, Yanofsky MF (1999) Interactions among APETALA1, LEAFY, and TERMINAL FLOWER1 specify meristem fate. Plant Cell **11:** 1007-1018

Lindermayr C, Sell S, Müller B, Leister D, Durner J (2010) Redox regulation of the NPR1-TGA1 system of *Arabidopsis thaliana* by nitric oxide. Plant Cell **22:** 2894-2907

Liu C, Chen H, Er HL, Soo HM, Kumar PP, Han J-H, Liou YC, Yu H (2008) Direct interaction of AGL24 and SOC1 integrates flowering signals in Arabidopsis. Development **135:** 1481-1491

Liu C, Zhou J, Drori-Bracha K, Yalovsky S, Ito T, Yu H (2007) Specification of Arabidopsis floral meristem identity by repressionof flowering time genes. Development **134:** 1901-1910

Lloyd AM, Schena M, Walbot V, Davis RW (1994) Epidermal cell fate determination in Arabidopsis: patterns defined by a steroid-inducible regulator. Science **266:** 436-439

Lodha M, Marco CF, Timmermans MCP (2013) The ASYMMETRIC LEAVES complex maintains repression of KNOX homeobox genes via direct recruitment of Polycomb-repressive complex2. Genes Dev **27:** 596-601

Long JA, Moan EI, Medford JI, Barton MK (1996) A member of the KNOTTED class of homeodomain proteins encoded by the *STM* gene of Arabidopsis. Nature **379:** 66-69

Maciejewska B, Kopceiwicz J (2002) Inhibitory effect of methyl jasmonate on flowering and elongation growth in *Pharbitis nil*. J Plant Growth Regul **21:** 216-223

Maciejewska BD, Kesy J, Zielinska M, Kopcewicz J (2004) Jasmonates inhibit flowering in short-day plant *Pharbitis nil*. Plant Growth Regul **43:** 1-8

Magome H, Yamaguchi S, Hanada A, Kamiya Y, Oda K (2008) The DDF1 transcriptional activator upregulates expression of a gibberellin-deactivating gene, GA2ox7, under high-salinity stress in Arabidopsis. Plant J **56:** 613-626

Magome H, Yamguchi S, Hanada A, Kamiya Y, Oda K (2004) Dwarf and delayed-flowering 1, a novel Arabidopsis mutant deficient in gibberellin biosynthesis because of overexpression of a putative AP2 transcription factor. Plant J **37:** 720-729

Marion J, Bach L, Bellec Y, Meyer C, Gissot L, Faure J-D (2008) Systematic analysis of protein subcellular localization and interaction using high-throughput transient transformation of Arabidopsis seedlings. Plant J **56:** 169-179

Mathieu J, Warthmann N, Kuttner F, Schmid M (2007) Export of FT protein from phloem companion cells is sufficient for floral induction in Arabidopsis. Curr Biol **17:** 1055-1060

Matsoukas I, Massiah AJ, Thomas B (2012) Florigenic and antiflorigenic signaling in plants. Plant Cell Physiol **53:** 1827-1842

McKim SM, Stenvik GE, Butenko MA, Kristiansen W, Cho SK, Hepworth SR, Aalen RB, Haughn GW (2008) The *BLADE-ON-PETIOLE* genes are essential for abscission zone formation in Arabidopsis. Development **135:** 1537-1546

Meyerowitz EM (1989) Arabidopsis, a useful weed. Cell **56:** 263-269

Michaels SD, Amasino RM (1999) *FLOWERING LOCUS C* encodes a novel MADS domain protein that acts as a repressor of flowering. Plant Cell **11:** 949-956

Michaels SD, Ditta G, Gustafson-Brown C, Pelaz S, Yanofsky M, Amasino RM (2003) AGL24 acts as a promoter of flowering in Arabidopsis and is positively regulated by vernalization. Plant J **33:** 867-874

Mutasa-Göttgens E, Hedden P (2009) Gibberellin as a factor in floral regulatory networks. J Exp Bot **60:** 1979-1989

Navarro L, Bari R, Achard P, Lisón P, Nemri A, Harberd NP, Jones JDG (2008) DELLAs control plant immune repsonses by modulating the balance of jasmonic acid and salicylic acid signaling. Curr. Biol. **16:** 650-655

Norberg M, Holmlund M, Nilsson O (2005) The *BLADE ON PETIOLE* genes act redundantly to control the growth and development of lateral organs. Development **132:** 2203-2213

Parcy F (2005) Flowering: a time for integration. Int J Dev Biol **49:** 585-593

Park J-H, Halitschke R, Kim BH, Baldwin IT, Feldmann K, Feyereisen R (2002) A knock-out mutation in allene oxide synthase results in male sterility and defective wound signal transduction in Arabidopsis due to a block in jasmonic acid biosynthesis. Plant J **31:** 1-12

Peaucelle A, Morin H, Traas J, Laufs P (2007) Plants expressing a miR164-resistant *CUC2* gene reveal the importance of post-meristematic maintenance of phyllotaxy in Arabidopsis. Development **135:** 1045-1050

Poethig RS (1990) Phase change and regulation of shoot morphogenesis. Science **250:** 923-930

Porri A, Torti S, Romera-Branchat M, Coupland G (2012) Spatially distinct regulatory roles for gibberellins in the promotion of flowering of Arabidopsis under long photoperiods. Development **139:** 2198-2209

Pose D, Yant L, Schmid M (2012) The end of innocence: flowering networks explode in complexity. Curr Opin Plant Biol **15:** 45-50

Proveniers M (2013) Sugars speed up the circle of life. eLife **2:** e00625

Proveniers M, Rutjens B, Brand M, Smeekens S (2007) The Arabidopsis TALE homeobox gene *ATH1* controls floral competency through positive regulation of *FLC*. Plant J **52:** 899-913

Ragni L, Belles-Boix E, Gunl M, Pautot V (2008) Interaction of KNAT6 and KNAT2 with BREVIPEDICELLUS and PENNYWISE in Arabidopsis inflorescences. Plant Cell **20:** 888-900

Rast MI, Simon R (2008) The meristem-to-organ boundary: more than an extremity of anything. Curr Opin Genet Dev **18:** 287-294

Ratcliffe OJ, Bradley DJ, Coen ES (1999) Separation of shoot and floral identity. Development **126:** 1109-1120

Rutjens B, Bao D, van Eck-Stouten E, Brand M, Smeekens S, Proveniers M (2009) Shoot apical meristem function in Arabidopsis requires the combined activities of three BEL1-like homeodomain proteins. Plant J **58:** 641-654

Sablowski R (2007) Flowering and determinacy in Arabidopsis. J Exp Bot **58:** 899-907

Samach A, Onouchi H, Gold E, Ditta GS, Schwarz-Sommer Z, Yanofsky MF, Coupland G (2000) Distinct role of CONSTANS target genes in reproductive development of Arabidopsis. Science **288:** 1613-1616

Schindler U, Beckman H, Cashmore AR (1992) TGA1 and G-Box binding factors: two distinct classes of Arabidopsis leucine zipper proteins compete for the G-Box-like element TGACGTGG. Plant Cell **4:** 1309-1319

Schoof H, Lenhard M, Haecker A, Mayer KFX, Jurgens G, Laux T (2000) The stem cell population of Arabidopsis shoot meristems is maintained by a regulatory loop between the *CLAVATA* and *WUSCHEL* genes. Cell **100:** 635-644

Schultz EA, Haughn WG (1991) *LEAFY*, a homeotic gene that regulates inflorescence development in Arabidopsis. Plant J **3:** 771-781

Schwechheimer C, Willige B (2009) Shedding light on gibberellic acid signaling. Curr Opin Plant Biol **12:** 57-62

Searle I, He Y, Turck F, Vincent C, Fornara F, Krober S, Amasino RM, Coupland G (2006) The transcription factor FLC confers a flowering repsonse to vernalization by repressing meristem competence and systemic signaling in Arabidopsis. Genes Dev **20:** 898-912

Serino G, Guiliana G (2011) *Arabidopsis thaliana* as an experimental organism. *In* Encylopedia of Life Sciences. John Wiley and Sons, Ltd., pp 1-10

Shearer HL, Cheng YT, Wang L, Liu J, Boyle P, Després C, Zhang Y, Li X, Fobert PR (2012) Arabidopsis clade I TGA transcription factors regulate plant defenses in an NPR1-independent fashion. Mol Plant Microb Interact **25:** 1459-1468

Sheldon CC, Burn JE, Perez PP, Metzger J, Edwards JA, Peacock WJ, Dennis ES (1999) The *FLF* MADS box gene: a repressor of flowering in Arabidopsis regulated by vernalization and methylation. Plant Cell **11:** 445-458

Shi CL, Stenvik GE, Vie AK, Bones AM, Pautot V, Proveniers M, Aalen RB, Butenko MA (2011) Arabidopsis class I KNOTTED-like homeobox proteins act downstream in the IDA-HAE/HSL2 floral abscission signaling pathway. Plant Cell **23:** 2553-2567

Shindo C, Aranzana MJ, Lister C, Baxter C, Nicholls C, Nordborg M, Dean C (2005) Role of FRIGIDA and FLOWERING LOCUS C in determining variation in flowering time of Arabidopsis. Plant Physiol **138:** 1163-1173

Sieburth LE, Meyerowitz EM (1997) Molecular dissection of the AGAMOUS control region shows that cis elements for spatial regulation are located intragenically. Plant Cell **9:** 355-365

Simpson GG (2004) The autonomous pathway: epigenetic and post-transcriptional gene regulation in the control of Arabidopsis flowering time. Curr Opin Plant Biol **7:** 570-574

Smith HM, Campbell BC, Hake S (2004) Competence to respond to floral inductive signals requires the homeobox genes *PENNYWISE* and *POUND-FOOLISH*. Curr Biol **14:** 812-817

Smith HM, Hake S (2003) The interaction of two homeobox genes, BREVIPEDICELLUS and PENNYWISE, regulates internode patterning in the Arabidopsis inflorescence. Plant Cell **15:** 1717-1727

Smith HM, Ung N, Lal S, Courtier J (2011) Specification of reproductive meristems requires the combined function of SHOOT MERISTEMLESS and floral integrators FLOWERING LOCUS T and FD during Arabidopsis inflorescence development. J Exp Bot **62:** 583-593

Somerville C, Koorneef M (2002) A fortunate choice: the history of Arabidopsis as a model plant. Nat Rev Genet **3:** 883-889

Song YH, Song NY, Shin SY, Kim HJ, Yun D-J, Lim CO, Lee SY, Kang KY, Hong JC (2008) Isolation of CONSTANS as a TGA4/OBF4 interacting protein. Mol. Cells **25:** 559-565

Sparkes IA, Runions J, Kearns A, Hawes C (2006) Rapid, transient expression of fluorescent fusion proteins in tobacco leaves and generation of stably transformed plants. Nat Protoc **4:** 2019-2025

Srikanth A, Schmid M (2011) Regulation of flowering time: all roads lead to Rome. Cell Mol Life Sci **68:** 2013-2037

Steeves TA, Sussex IM (1989) Patterns in plant development. Academic, New York

Stief A, Altmann S, Hoffmann K, Pant BD, Scheible W-R, Baurle I (2014) Arabidopsis miR156 regulates tolerance to recurring environmental stress through SPL transcription factors. Plant Cell **26:** 1792-1807

Sun S, Yu J-P, Chen F, Zhou T-J, Fang X-H, Li Y-Q, Sui S-F (2008) TINY, a dehydration-responsive element (DRE)-binding protein-like transcription factor connecting the DRE- and ethylene-responsive element-mediated signaling pathways in Arabidopsis. J Biol Chem **283:** 6261-6271

Tabb P (2012) Genetic analysis of co-ordination of flowering and regulation of inflorescence architecture in *Arabidopsis thaliana*. Carleton University, Ottawa

Takeda S, Aida M (2011) Establishment of the embryonic shoot apicall meristem in *Arabidopsis thaliana*. J Plant Res **124:** 211-219

Telfer A, Bollman KM, Poethig RS (1997) Phase change and the regulation of trichome distribution in *Arabidopsis thaliana*. Development **124:** 645-654

Teper-Bamnolker P, Samach A (2005) The flowering integrator FT regulates SEPALLATA3 and FRUITFULL accumulation in Arabidopsis leaves. Plant Cell **17:** 2661-2675

Turck F, Fornara F, Coupland G (2008) Regulation and identity of florigen: FLOWERING LOCUS T moves center stage. Annu Rev Plant Biol **59:** 573-594

Ung N, Lal S, Smith HM (2011) The role of PENNYWISE and POUND-FOOLISH in the maintenance of the shoot apical meristem in Arabidopsis. Plant Physiol **156:** 605-614

Vionnet O, Rivas S, Mestre P, Baulcombe D (2003) An enhanced transient expression systems in plants based on suppression of gene silencing by the p19 protein of tomato bushy stunt virus. Plant J **33:** 949-956

Vroemen CW, Mordhorst AP, Albrecht C, Kwaaitaal MACJ, de Vries SC (2003) The *CUP-SHAPED COTYLEDON3* gene is required for boundary and shoot meristem formation in Arabidopsis. Plant Cell **15:** 1563-1577

Wagner D, Sablowski RWM, Meyerowitz EM (1999) Transcriptional activation of APETALA1 by LEAFY. Science **285:** 582-584

Wahl V, Ponnu J, Schlereth A, Arrivault S, Langenecker T, Franke A, Feil R, Lunn JE, Stitt M, Schmid M (2013) Regulation of flowering by trehalose-6-phosphate signaling in *Arabidopsis thaliana*. Science **339:** 704-707

Wang J-W, Czech, B., and Weigel. D. (2009) miR156-regulated SPL transcription factors define an endogenous flowering pathway in *Arabidopsis thaliana*. Cell **138:** 738-739

Wang JW (2014) Regulation of flowering time by the miR156-mediated age pathway. J Exp Bot **65:** 4723-4730

Werner JD, Borevitz JO, Uhlenhaut NH, Ecker JR, Chory J, Weigel D (2005) FRIGIDA-independent variation in flowering time of natural *Arabidopsis thaliana* accessions. Genetics **170:** 1197-1207

Wigge PA, Kim MC, Jaeger KE, Busch W, Schmid M, Lohmann JU, Weigel D (2005) Integration of spatial and temporal information during floral induction in Arabidopsis. Science **309:** 1056-1059

Wild M, Achard P (2013) The DELLA protein RGL3 positively contributes to jasmonate/ethylene defense responses. Plant Signal Behav **8:** e23891

Wild M, Daviere JM, Cheminant S, Regnault T, Baumberger N, Heintz D, Baltz R, Genschik P, Achard P (2012) The Arabidopsis DELLA RGA-LIKE3 is a direct target of MYC2 and modulates jasmonate signaling responses. Plant Cell **24:** 3307-3319

William RF (1974) The shoot apex and leaf growth: a study in quantitative biology. Cambridge University Press, London

Wingler A, Delatte TL, O'Hara LE, Primavesi LF, Jhurreea D, Paul MJ, Schleupmann H (2012) Trehalose 6-phosphate is required for the onset of leaf senescence associated with high carbon availability. Plant Physiol **158:** 1241-1251

Wolpert L, Tickle C, Lawrence P, Meyerowitz EM, Robertson E, Smith J, Jessel T (2011) Principles of Development, Ed 4th. Oxford University Press, New York

Wu G, Park M, Conway S, Wang J-W, Weigel D, Poethig RS (2009) The sequential action of miR156 and miR172 regulates developmental timing in Arabidopsis. Cell **138:** 750-759

Wu G, Poethig RS (2006) Temporal regulation of shoot development in *Arabidopsis thaliana* by miR156 and its target SPL3. Development **133:** 3539-3547

Wu Y, Zhang D, Chu JY, Boyle P, Wang Y, Brindle ID, De Luca V, Després C (2012) The Arabidopsis NPR1 protein is a receptor for the plant defense hormone salicylic acid. Cell Rep **1:** 639-647

Xie D-X, Feyes BF, James S, Nieto-Rostro M, Turner JG (1998) *COI1*: an Arabidopsis gene required for jasmonate-regulated defense and fertility. Science **280:** 1091-1094

Xu M, Hu T, McKim SM, Murmu J, Haughn GW, Hepworth SR (2010) Arabidopsis BLADE-ON-PETIOLE1 and 2 promote floral meristem fate and determinacy in a previously undefined pathway targeting APETALA1 and AGAMOUS-LIKE24. Plant J **63:** 974-989

Yadav RK, Perales M, Gruel J, Girke T, Jonsson H, Reddy GV (2011) WUSCHEL protein movement mediates stem cell homeostasis in the Arabidopsis shoot apex. Genes Dev **25:** 2025-2030

Yamaguchi A, Abe M (2012) Regulation of reproductive development by non-coding RNA in Arabidopsis: to flower or not to flower. J Plant Res **125:** 693-704

Yamaguchi A, Wu MF, Yang L, Wu G, Poethig RS, Wagner D (2009) The microRNA-regulated SBP-Box transcription factor SPL3 is a direct upstream activator of LEAFY, FRUITFULL, and APETALA1. Dev Cell **17:** 268-278

Yan J, Zhang C, Gu M, Bai Z, Zhang W, Qi T, Cheng Z, Peng W, Luo H, Nan F, Wang Z, Xie D (2009) The Arabidopsis CORONATINE INSENSITIVE1 protein is a jasmonate receptor. Plant Cell **21:** 2220-2236

Yang D-L, Yao J, Mei C-S, Tong X-H, Zeng L-J, Li Q, Xiao L-T, Sun T, Li J., Deng X-W, Lee CM, Thomashow MF, Yang Y., He Z, He SY (2012) Plant hormone jasmonate prioritizes defense over growty by interfering with gibberellin signaling cascade. Proc. Natl. Acad. Sci. USA **109**

Yang L, Xu M, Koo Y, He J, Poethig RS (2013) Sugar promotes vegetative phase change in Arabidopsis thaliana by repressing the expression of MIR156A and MIR156C. eLife **2:** e00260

Yant L, Mathieu J, Schmid M (2009) Just say no: floral repressors help Arabidopsis bide the time. Curr Opin Plant Biol **12:** 580-586

Yu H, Ito T, Wellmer F, Meyerowitz EM (2004) Repression of *AGAMOUS-LIKE 24* is a crucial step in promoting flower development. Nat Genet **36:** 157-161

Yu S, Cao L, Zhou C-M, Zhang T-Q, Lian H, Sun Y, Wu J, Huang J, Wang G, Wang J-W (2013) Sugar is an endogenous cue for juvenile-to-adult phase transition in plants. eLife **2:** e00269

Yu S, Galvao VC, Zhang YC, Horrer D, Zhang TQ, Hao YH, Feng YQ, Wang S, Schmid M, Wang JW (2012) Gibberellin regulates the Arabidopsis floral transition through miR156-targeted SQUAMOSA promoter binding-like transcription factors. Plant Cell **24:** 3320-3332

Zhang Y, Tessaro MJ, Lassner M, Li X (2003) Knockout analysis of Arabidopsis transcription factors TGA2, TGA5, and TGA6 reveals their redundant and essential roles in systemic acquired resistance. Plant Cell **15:** 2647-2653

Zhang Y, Turner JG (2008) Wound-induced endogenous jasmonates stunt plant growth by inhibiting mitosis. PLoS One **3:** e3699

Zhu Q-H, Helliwell CA (2011) Regulation of flowering time and floral patterning by miR172. J Exp Bot **62:** 487-495

APPENDIX I

Gene ontology classification of differentially expressed genes in *bop1-6D* versus wild-type internode microarrays.

	GO Term	**Description**	**Number of genes**	**p-value**
	Upregulated in *bop1-6D*		n=3158 total	
1	GO:0050896	response to stimulus	633	6.8e-59
2	GO:0042221	response to chemical stimulus	385	5.6e-49
3	GO:0010033	response to organic substance	272	2.1e-40
4	GO:0006950	response to stress	381	2.9e-38
5	GO:0009607	response to biotic stimulus	165	5.8e-35
6	GO:0051707	response to other organism	155	6.4e-33
7	GO:0010200	response to chitin	73	5.4e-29
8	GO:0051704	multi-organism process	167	1.8e-27
9	GO:0009743	response to carbohydrate stimulus	81	1.1e-21
10	GO:0009719	response to endogenous stimulus	191	7.2e-23
11	GO:0006952	defense response	150	2.5e-21
12	GO:0009617	response to bacterium	72	2.6e-18
13	GO:0009753	response to jasmonic acid stimulus	61	2.8e-15
14	GO:0009725	response to hormone stimulus	157	3.5e-15
15	GO:0009611	response to wounding	57	1.1e-14
	Downregulated in *bop1-6D*		n=3174 total	
1	GO:004426	cellular carbohydrate metabolic process	107	3.9e-24

2	GO:0008152	metabolic process	1030	6.2e-21
3	GO:0005975	carbohydrate metabolic process	157	4.8e-21
4	GO:0009791	post-embryonic development	137	8.9e-21
5	GO:0009987	cellular process	1098	2.5e-14
6	GO:0034641	cellular nitrogen compound metabolic process	104	1.6e-17
7	GO:0006629	lipid metabolic process	138	1.6e-15
8	GO:0042180	cellular ketone metabolic process	142	2.4e-15
9	GO:0008610	lipid biosynthetic process	89	6.9e-15
10	GO:0043436	oxoacid metabolic process	138	6.8e-15
11	GO:0006082	organic acid metabolic process	138	7.4e-15
12	GO:0019752	carboxylic acid metabolic process	138	6.8e-15
13	GO:0044238	primary metabolic process	854	2.5e-14
14	GO:0016043	cellular component organization	168	1.0e-13
15	GO:0006066	alcohol metabolic process	61	2.5e-12