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STOMATAL CARPENTER 1 controls stomata development by affecting SPEECHLESS activity

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Abstract

Stomata consist of two specialised epidermal cells termed guard cells (GCs) surrounding a pore, through which gas exchange can occur. In *Arabidopsis thaliana*, a dedicated cell lineage is initiated and undergoes a series of cell divisions to produce a stoma. A set of basic helix-loop-helix (bHLH) transcription factors regulates the differentiation events through the lineage. The initiation and proliferation of stomatal lineage cells is controlled by the transcription factor SPEECHLESS (SPCH), which drive the asymmetric division of meristemoid mother cell to originate a small triangular cell termed meristemoid. Which transcriptional events precede stomata lineage specification and are required for correct GC patterning, however, remain unclear. The DOF transcription factor *STOMATAL CARPENTER 1* (*SCAP1*) has previously shown to be expressed in GC and involved in stomata function, by activating a set of GC–specific genes required for GC maturation and activity. We show that *SCAP1* expression can also be observed in young leaf primordia, before any GC differentiation occurs. The study of transgenic plants carrying a *proSCAP1:GUS-GFP* transcriptional fusion coupled with quantitative PCR analyses indicate that *SCAP1* expression is maximal in a stomatal lineage competence domain, coincident with *SPCH* expression. We found that SCAP1 modulates stomata development; independent *scap1* loss-of-function mutants show a reduced number of GCs whilst *SCAP1* over expression lines have an increased number of GCs in addition to altered GC distribution and spacing patterns. Confocal imaging of SPCH-GFP protein in a background carrying inducible *SCAP1* shows that SCAP1 activation results in an increased number of nuclei expressing SPCH-GFP. Our results suggest an early role for *SCAP1* in GC differentiation through SPCH protein stabilization. *SCAP1* may thus link different aspects of GC biology including specification, maturation and function.

State of the Art

Stomata are microscopic pores on the epidermal surface of land plants, with a crucial role in regulating transpiration and gas exchange between the plant and atmosphere. Stomata consist of two specialised epidermal cells termed guard cells (GCs) surrounding a pore. Changes in turgor pressure in GCs cause the opening or closure and determine the amount of gas exchanges. In the model plant *Arabidopsis thaliana*, stomata are present on the epidermis of all above-ground organs except for petals and stamen filaments (Geisler, Yang, & Sack, 1998; Pillitteri, Bogenschutz, & Torii, 2008; Robinson et al., 2011; Sessions et al., 1997). Stomata are produced post-embryonically and are distributed across the epidermis in an organ–specific manner. The number and placement of stomata are genetically regulated and also affected by the environment (Hetherington & Woodward, 2003; Nadeau & Sack, 2002). Alterations in stomatal distribution affects CO2 uptake, evaporation, and internal leaf temperature in much the same way as pore opening and closing (Yoo, Hasegawa, & Mickelbart, 2011). Therefore, control of stomata placement is a key trait for plant survival and fitness.

Stomata development in Arabidopsis

Stomata development and placement are regulated by complex regulatory networks that incorporate several events, including hormonal and environmental cues. The pathways controlling stomata formation are best understood in Arabidopsis. Stomata are produced by a dedicated and specialized cell lineage (Nadeau & Sack, 2003; Zhao & Sack, 1999). This lineage is prevalent in the developing organs and is inactive after epidermal maturation. During the early stages of leaf development, undifferentiated epidermal cells of leaf primordia (termed protodermal cells, PDCs) either differentiate into pavement cells (PC) or acquire the competence to initiate the stomatal cell lineage and become meristemoid mother cells (MMCs). The meristemoid mother cell undergoes a series of cell divisions and successive identity changes (Figure 1). These transitions are characterized by changes in cell morphology, and associated with alterations in their transcriptomic signature (Dong, MacAlister, & Bergmann, 2009; Geisler et al., 2000; Pillitteri & Dong, 2013). Each MMC divides asymmetrically to produce the first specialized cells of the stomatal cell lineage, a small triangular cell termed meristemoid. These divisions produce a larger cell called a stomatal-lineage ground cell (SLGC) that can differentiate into a lobed pavement cell or has the potential to divide asymmetrically to produce satellite meristemoids and their sister cell (Bergmann & Sack, 2007; Geisler et al., 2000; Nadeau, 2009). Each meristemoid can undergo one or two round of asymmetric cell divisions, which result in another meristemoid and a sister cell. The meristemoid eventually

differentiates into a round guard mother cell (GMC), which divides symmetrically to generate two paired guard cells surrounding the stomata pore (Figure1).

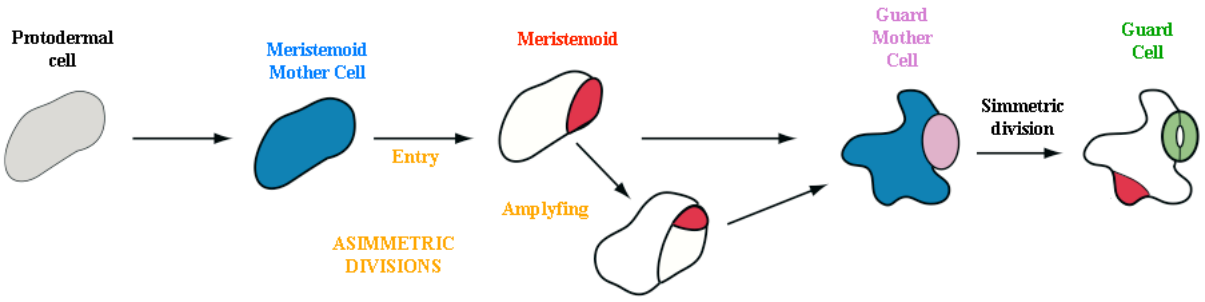

Figure 1 - *Diagram of key stages and divisions in Arabidopsis thaliana stomatal development*.
Protodermal cells in the epidermis are converted into meristemoid mother cells (MMCs) through an unknown process. MMCs undergo an asymmetric entry division to create a meristemoid. Meristemoids may undergo additional asymmetric amplifying divisions, or convert into a guard mother cell (GMC). The GMC will divide a single time, symmetrically, to form the two guard cells. Cells next to meristemoids, GMCs, and guard cells can become MMCs and undergo spacing divisions to create new meristemoids. The plane of this division is oriented so that the new meristemoid is placed away from the pre-existing stoma or precursor cell (image modified from Bergmann & Sack, 2007).

Specification of the stomatal cell lineage

The genes responsible for GCs specification and development have been characterised. Specification of the MMC, GMC and GC identities is governed by three closely related basic helix-loop-helix (bHLH) transcription factors (TFs) *SPEECHLESS* (*SPCH*), *MUTE*, and *FAMA*, respectively (Figure 2) (MacAlister, Ohashi-Ito, & Bergmann, 2007; Ohashi-Ito & Bergmann, 2006; Pillitteri, Sloan, Bogenschutz, & Torii, 2007). Two other bHLH protein, SCREAM/ICE1 and SCREAM2, act redundantly together with SPCH, MUTE, and FAMA via the formation of heterodimers (Figure 2) (Kanaoka et al., 2008). The bHLH family contains at least 158 members in Arabidopsis (Pires & Dolan, 2010). It can be subdivided into 26 sub-groups on the base of other distinctive functional and conserved domains. *SPCH*, *MUTE*, and *FAMA* form a single clade, that comprises bHLH subgroup Ia whereas ICE1/SCRM, and SCRM2, are members of group IIIb clade (Heim et al., 2003; MacAlister & Bergmann, 2011; Ohashi-Ito & Bergmann, 2006; Pires & Dolan, 2010). These five genes drive the progression of the stomatal lineage (Figure 2). *SPCH* promotes an asymmetric division of MMC to initiate the stomatal lineage (MacAlister et al., 2007). MUTE is required to terminate asymmetric divisions and for the transition to GMC (Pillitteri et al., 2007). FAMA controls the final transition from GMC to GC (Ohashi-Ito & Bergmann, 2006). Loss of

function in any of these genes results in no functional GCs (Figure 3). Contrariwise, their over expression results in an ectopic formation of GCs (Figure 3) (Pillitteri & Torii, 2012).

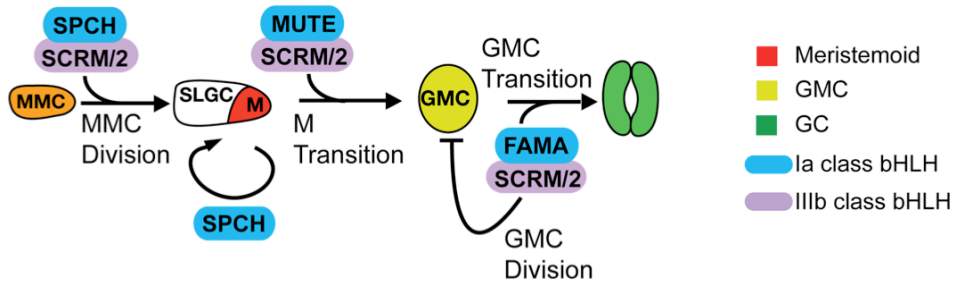

Figure 2 - *Diagram of the actions of the stomatal bHLH proteins.*
SPCH and SCRM/2 direct MMC transition, MUTE and SCRM/2 direct the GMC transition and FAMA and SCRM/2 promote the GC transition (image modified from Pillitteri & Dong, 2013).

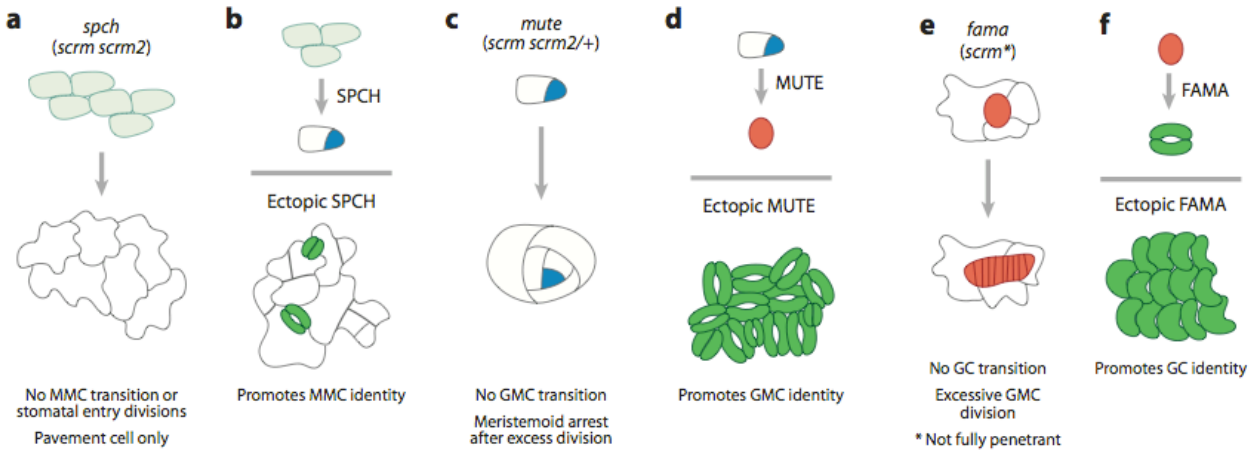

Figure 3 - *Epidermal phenotypes due to altered activities of transcription factor genes regulating stomatal cell-state transitions.* (a) *spch* and *scrm scrm2* double mutants produce identical phenotypes consisting of pavement cells only. (b) Ectopic *SPEECHLESS* (*SPCH*) induces stomatal entry divisions producing a highly divided epidermis. (c) *mute* and *scrm scrm2/+* plants produce similar phenotypes resulting in meristemoid (dark blue) arrest. (d) *MUTE* overexpression causes all epidermal cells to become guard mother cells (GMCs) (red) and differentiate into stomata. (e) *fama* and *scrm* both display additional GMC divisions, although only *fama* produces an absolute block in guard-cell (GC) (green) transition. (f) *FAMA* overexpression results in the production of single GCs, consistent with its role in inhibiting GMC division (image modified from Pillitteri & Torii, 2012).

SPCH is key switch of stomatal lineage initiation

SPCH protein promotes MMCs identity (MacAlister et al., 2007; Pillitteri et al., 2007). *SPCH* activity regulates the number of MMC-to-meristemoid transitions during stomatal cell lineage specification. As a result of a complete lack of stomatal-lineage initiation, *spch* loss-of-function mutants produce an epidermis composed solely of interlocking pavement cells (Figure 3a) (MacAlister et al., 2007). The mutation *spch* significantly reduces the number of SLGC associated

with mature stomata, indicating that *SPCH* promotes meristemoid asymmetric amplifying divisions to increase the number of SLGCs (Lampard, MacAlister, & Bergmann, 2008; MacAlister et al., 2007; Robinson et al., 2011). As the frequency of asymmetric cell divisions is the major determinant of the number of both stomatal and non-stomatal epidermal cell, cell density can be regulated by modulation of SPCH activity (Figure 5) (Lampard et al., 2008; MacAlister et al., 2007).

Experiments utilising *SPCH* promoter-reporter transcriptional fusions revealed that *SPCH* is expressed in the developing leaf epidermis and then persists in the stomatal lineage cells (GMC and GCs). However, SPCH protein can only been detected in undifferentiated PDCs and MMCs, suggesting that *SPCH* is regulated at the post-transcriptional level (MacAlister et al., 2007). *SPCH* expression is often found in two neighbouring cells, a pattern consistent with its role in the dividing cell population (Figure 4). In older organs, *proSPCH:SPCH–GFP* expression is restricted to small cells in the epidermis, including cells that have recently divided next to stomatal lineage cells (Figure 4) (MacAlister et al., 2007).

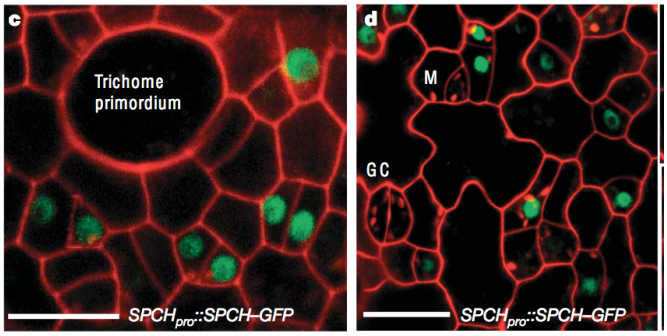

Figure 4 - *SPCH protein expression pattern.*
SPCHpro::SPCH–GFP in the epidermis of developing cotyledons and leaves before (c) and during (d) development of the stomatal lineage, and during spacing divisions. Cell outlines were counterstained with propidium iodide (red); M indicates meristemoid, GC marks guard cells (image from MacAlister et al., 2007).

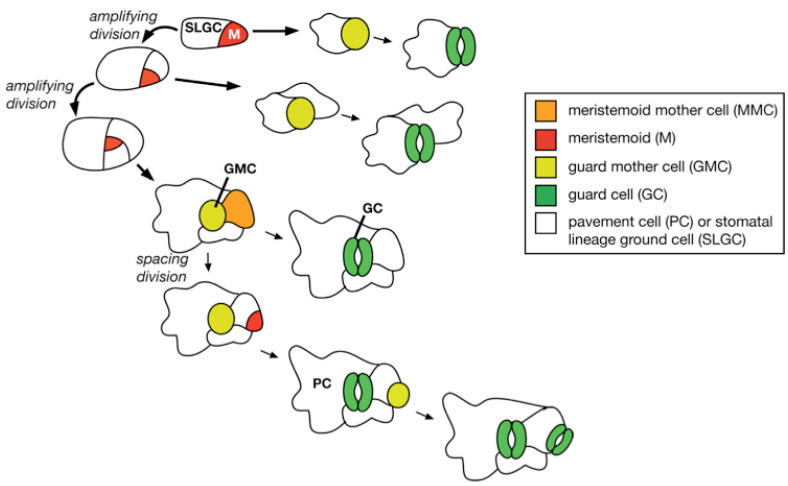

Figure 5 - *Diagram of stomatal lineage progression in Arabidopsis.*
A subset of protodermal cells (*grey*) becomes meristemoid mother cells (MMC, orange). MMCs enter the stomatal lineage through an asymmetric division to create two daughter cells, a meristemoid (M, *red*) and a stomatal-lineage ground cell (SLGC). Meristemoids can undergo a limited number of amplifying divisions, but eventually transition into a guard mother cell (GMC, *yellow*). An SLGC can differentiate into a pavement cell (PC) or attain MMC identity to produce a satellite meristemoid positioned away from an existing stomatal precursor. A GMC divides once symmetrically to produce two equally-sized guard cells (GCs, green)(image modified from Pillitteri et al., 2013).

One-Cell Spacing Rule

Several lines of evidence indicate that cell-cell communication is crucial in stomatal patterning and several signalling components have been identified, which include the ERECTA leucine-rich repeat receptor like kinases (LRR- RLKs) (ER and ERL), an LRR receptor-like protein (TMM), MAP kinase components (YODA, MKK and MPK*)* and a family of small cysteine-rich secreted peptides (EPFs and EPFL). The one-cell-spacing rule dictates that new meristemoids will be oriented at least one cell away from existing stomatal precursors (Geisler et al., 2000; Hara, Kajita, Torii, Bergmann, & Kakimoto, 2007; Hara et al., 2009; Hunt & Gray, 2009; Shpak, McAbee, Pillitteri, & Torii, 2005). Correct spacing is required to ensure efficient stomatal opening and closure and gas exchange regulation.

Cell-Cell Signalling Components

Stomata are produced throughout leaf development, and many form by division of cells next to pre-existing stomata but the division is oriented so that the satellite meristemoid does not contact the previous one (spacing division) (Figure5) (Berger & Altmann, 2000; Geisler et al., 2000). The orientation of cell divisions is controlled by different types of intercellular signalling mechanisms. Both positive and negative regulators of stomatal density have been characterized. The ERECTA

(ER) family of leucine-rich repeat (LRR) receptor–like kinases and the LRR receptor–like protein TOO MANY MOUTHS (TMM) are positive regulators of stomata spacing (Geisler et al., 2000; Hara et al., 2007; Hunt & Gray, 2009; Hunt, Bailey, & Gray, 2010; J. S. Lee et al., 2012; Masle, Gilmore, & Farquhar, 2005; Nadeau & Sack, 2002; Shpak et al., 2005). The *MITOGEN ACTIVATED KINASE* (*MAPK*) genes act downstream of the LRR receptors and include *YODA*, *MKK4*/*MKK5* and *MPK3*/*MPK6* (Bergmann, Lukowitz, & Somerville, 2004; Lampard et al., 2008; Lampard, Lukowitz, Ellis, & Bergmann, 2009). The ER and ERL receptors perceive small secreted cysteine-rich peptides, acting as intercellular signals, which comprise *EPIDERMAL PATTERNING FACTOR* (*EPF*) and *EPIDERMAL PATTERNING FACTOR-LIKE* (*EPFL*). The EPF protein family (EPFf) participate in diverse developmental processes but has been demonstrated that, four members affect stomatal development, *EPF1, EPF2*, *CHALLAH* (*CHAL/EPFL6*), and *STOMAGEN*/EPFL9. Only *EPF1* and *EPF2* are specifically expressed in the stomatal lineage. *EPF2* is express in *SPCH*-expressing protodermal cells (MMCs) early in the lineage, whereas *EPF1* expression occurs at later-stages of meristemoid development, GMCs and young guard cells; these two peptides inhibit SPCH activity and prevent the development of stomata close to one another, thus effectively promoting and enforcing the one-cell spacing rule (Hara et al., 2007; 2009; Hunt & Gray, 2009). In contrast to *EPF1* and *EPF2*, *STOMAGEN* and *CHAL* influence stomatal development through inter-tissue signalling. *CHAL* is expressed in the hypocotyl while *STOMAGEN* expression occurs in the mesophyll. Both *CHAL* and *STOMAGEN* promote the production of stomata (Kondo et al., 2010; Sugano et al., 2010). The competition between the different EPFf peptides for the binding to the same receptors is thought to provide robustness to the patterning of stomata (Ohki, Takeuchi, & Mori, 2011). The effect of EPF1 and EPF2 perception lead to the activation of *MAPK* cascade that eventually modulates SPCH activity. SPCH protein contains a unique MAPK phosphorylation target domain that regulates its activity in vivo. SPCH phosphorylation cause its inactivation by degradation (Jewaria et al., 2013; Lampard et al., 2008).

Hormones and Environmental signals

Besides the EPFs, the LRR receptors and the MAPKs cascade, other signalling pathways play a role in stomata development. These pathways translate environmental cues and hormone signalling into affecting stomatal density in developing leaves.

- **Light** - Plants grown under high light intensity produce more stomata than those grown under normal light condition. Further, different wavelengths of light have contrasting effects

on stomatal density. High intensity red light increases stomatal density, whereas far-red treatment (shadow mimic) decreases it (Boccalandro et al., 2009; Casson et al., 2009; C.-Y. Kang, Lian, Wang, Huang, & Yang, 2009). Variations of light quality and intensity are perceived by photoreceptors CRY1, CRY2, PHYB, and PHYA and influence stomatal development through negative regulation of COP1 (CONSTITUTIVE PHOTOMORPHOGENIC 1) which acts upstream of YODA (Casson et al., 2009; C.-Y. Kang et al., 2009; H. Q. Yang, Tang, & Cashmore, 2001).

- **CO_2** - CO_2 regulates both stomata opening and development (Gray et al., 2000; Hashimoto et al., 2006). Several plant species produce fewer stomata in response to increased levels in atmospheric CO_2 (Woodward, 1987). Genes involved in CO_2 sensing and stomata development regulation have been characterised. Mutation in the *HIGH CARBON DIOXIDE* (*HIC*), encoding an enzyme involved in the production of cuticular wax polymers, result in more stomata than wild type under elevated CO2 concentrations (Gray et al., 2000). Also, two β-carbonic anhydrase CA1 and CA4 (βCA), CO_2-binding proteins, have a role in CO_2-induced stomatal closure and in early CO_2 signalling. The double mutant *ca1 ca4* displays increased stomatal density when grown under high CO_2 levels (Hu et al., 2010). Despite βCA1 and βCA4 are expressed in mesophyll and GCs, expression of either βCA1 or βCA4 in GCs is sufficient to complement the stomatal-density phenotype (Engineer et al., 2014). This observation suggests that GC-specific expression of βCA1 and βCA4 is important for the production of long-distance signals involved in stomata density regulation in response to CO_2 (Engineer et al., 2014; Hu et al., 2010). Long-range signals have been hypothesised to regulate stomatal development. When mature leaves are exposed to high CO_2 levels the emerging young leaves show reduced stomatal density (Lake et al., 2001; Lake, Woodward, & Quick, 2002). Therefore, a long-range signal must transduce the information from mature leaves to emerging leaves. EPF2 is essential for CO_2 control of stomatal development. Increased levels of *EPF2* transcript were found in wild type plants exposed to high CO_2 (Engineer et al., 2014). A protease called CRSP (CO2 RESPONSE SECRETED PROTEASE) cleaves and activates EPF2. Expression of CRSP depends on βCA1 and βCA4 and positively responds to elevated CO2 concentration. Thus, stomatal density is controlled by CO_2-dependent modulation of *CRSP* expression that positive regulates EPF2 activity by cleaving it (Engineer et al., 2014).

- **Brassinosteroids** - Brassinosteroids (BRs) are phytohormones involved in many developmental and physiological processes including stomatal development (T.-W. Kim, Michniewicz, Bergmann, & Wang, 2012). Under high BR levels, BIN2 is inactivated by phosphorylation (Mora-García et al., 2004). BRs negatively regulate stomatal development by inhibiting BIN2 kinase-mediated inhibition of YODA (T.-W. Kim et al., 2012) but also BIN2-dependent SPCH phosphorylation independent of the MAPKs (Gudesblat et al., 2012). MKK4/5, the downstream effector of YDA, are also targeted and inhibited by BIN2 phosphorylation (Khan et al., 2013).

- **Abscisic acid (ABA)** - Drought is detrimental for plant growth and survival. Low water conditions compromise gas exchanges as plants reduce stomata opening to minimize the water loss. An adaptive plant response to drought condition is to reduce the production of stomata during leaf development. The phytohormone ABA is produced in response to drought and regulates both stomata closure and stomata development. ABA-deficient mutants have an increased number of stomata as a result of increased *SPCH* and *MUTE* expression levels. Thus ABA acts upstream of these genes (Tanaka et al., 2013). An interesting aspect of the role of ABA in stomatal development is that ABA regulates both stomatal development and physiology.

DOF transcription factors can act as activators and repressors of transcription

Besides the bHLHs, other transcription factors may play an important role in GCs specification. The DOF (DNA-binding One Zinc Finger) family is an important class of transcriptional regulators restricted in their distribution to plants, and in *Arabidopsis thaliana* comprises 36 members (Riechmann et al., 2000) (Figure 6). DOF factors are expressed in most if not all tissues and act frequently in a functionally redundant manner. These transcription factors are involved in several fundamental aspects of plant development including, cell cycle regulation, seed development and germination, and flower induction (Noguero, Atif, Ochatt, & Thompson, 2013; Yanagisawa, 2002) (Figure 7). These transcription factors share a highly conserved DOF domain that consists of a region containing the amino acid string $CX_2CX_{21}CX_2C$, which binds zinc. This motif is essential for DNA binding and mutation of any of the four cysteines abolishes DNA binding. DOFs bind to their promoter targets to the consensus sequence AAAG (Kisu, Ono, Shimofurutani, Suzuki, & Esaka, 1998) although DNA binding specificity is also influenced by sequences flanking the AAAG core binding site (Yanagisawa & Schmidt, 1999). DOFs are nuclear localised and present a

transcriptional activation domain at their C-terminus (Yanagisawa, 2001). However, the regions N- and C-terminal to the DOF domain are variable among different DOF proteins. These regions may interact with different regulatory proteins or intercept signals that mediate activation or repression of gene expression. These interactions are likely to contribute to the diverse functions of DOF domain proteins (Yanagisawa, 2001) (Figure 6, 7).

DOF factors can either activate or repress transcription, depending on the type of DOF factor and the genomic context where they act. For example, *AtDOF5.1* activates expression of *REVOLUTA* (*REV*), a class III HD- ZIP transcription factor required for establishment of leaf adaxial-abaxial polarity (H.-S. Kim et al., 2010). In contrast *CYCLIN DOF FACTOR1* (*CDF1*) acts as a transcriptional repressors of *CONSTANS* (*CO*), a master regulator of photoperiodic flowering (Fornara et al., 2009). DOF genes belonging to the same clade can also have contrasting effects on transcription (Gualberti et al., 2002). The *DAG1* gene (*DOF AFFECTING GERMINATION*) reduces seed dormancy (Papi et al., 2000). A related DOF gene, *DAG2*, has an opposite effect, promoting dormancy (Gualberti et al., 2002). It was shown that *DAG1* and *2* act antagonistically on the same set of target promoters, with DAG1 repressing transcription and DAG2 activating.

DOF proteins regulate transcription by forming multimeric complexes with other transcription factors (Yanagisawa, 1997; Yanagisawa & Schmidt, 1999). *AtDOF4.7*, expressed in the floral organ abscission zone, physically interacts with a member of another Zn-finger TF family, AtZFP2, also implicated in repression of floral organ abscission (Wei et al., 2010). Another DOF, a negative regulator of seed germination, AtDOF6, putatively exerts its effect by interacting with TCP14, a positive regulator of seed germination (Rueda-Romero, Barrero-Sicilia, Gómez-Cadenas, Carbonero, & Oñate-Sánchez, 2012).

Possible role of DOF transcription factor in stomata development

Numerous publications indicate an involvement of DOF transcription factors in the regulation of guard cell development and function. *StDOF1* was identified as a candidate for activation of guard cell-specific expression. It interacts with and activates expression of the guard cell-specific gene *KST1* (Plesch, Ehrhardt, & Mueller-Roeber, 2001). DOF factors were proposed as prime candidates in regulating *AtMYB60* expression a guard cell-specific expressed transcription factor since deletions of putative DOF binding sites in its promoter decrease *AtMYB60* transcription (Cominelli et al., 2011). Recently the DOF transcription factor *SCAP1* has been shown to directly bind *AtMYB60* promoter and to positive regulate its transcription. *SCAP1* also regulates essential processes related to guard cell maturation and function. Mutants of *scap1* show transcript

alterations in multiple genes directly involved in stomatal movement and furthermore are defective in some cell wall mechanical properties of GCs (Negi et al., 2013). However, the potential role of *SCAP1* in stomata patterning has not previously been investigated.

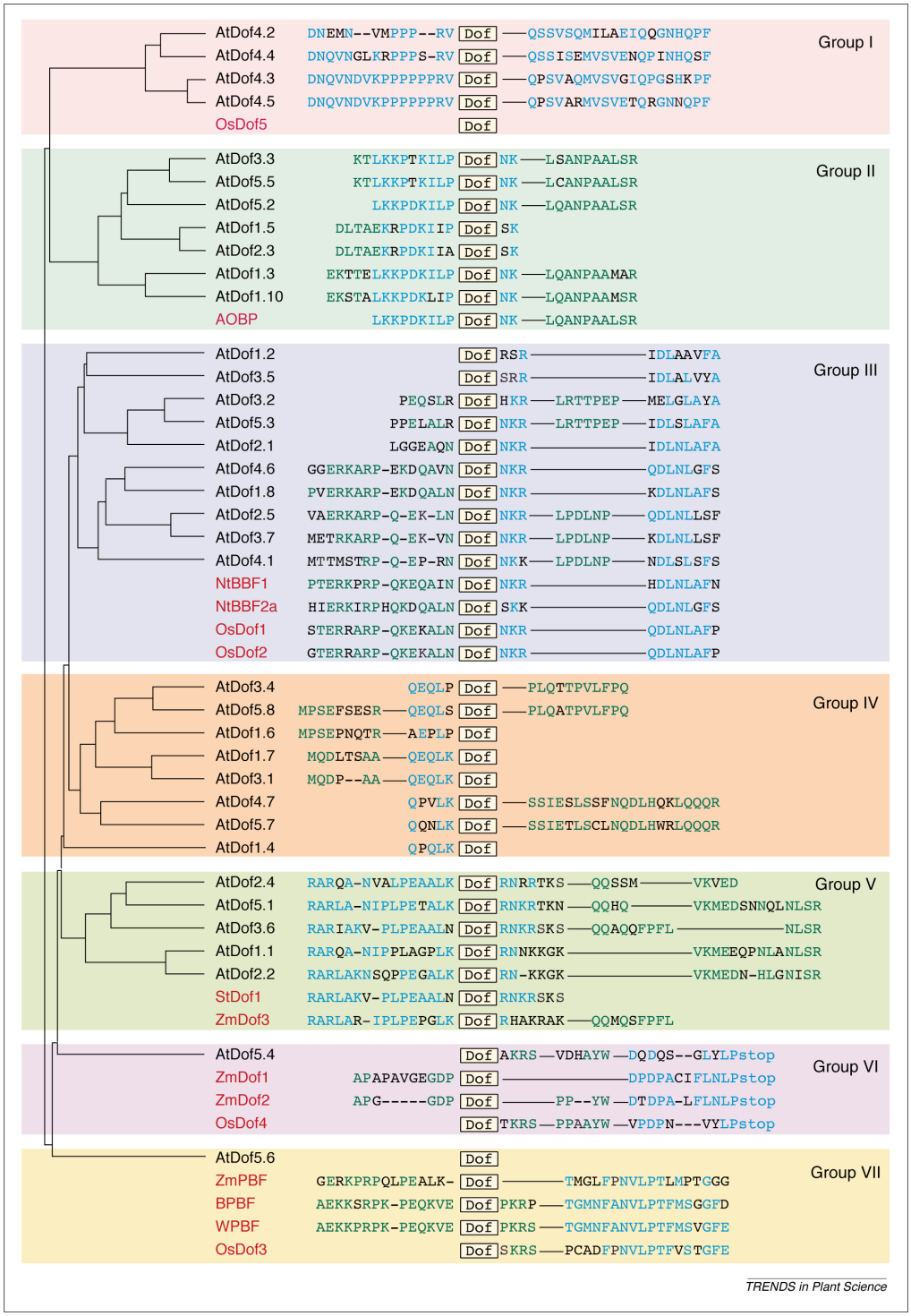

Figure 6 - *The phylogenetic tree shows the Dof protein relationships.*
The first one or two letters of the names indicate the initials of the species (except that of pumpkin, which is AOBP): At, Arabidopsis thaliana; B, barley; Nt, Nicotiana tabacum; Os, Oryza sativa; St, Solanum tuberosum; W, wheat, Zm, Zea mays. The signature motifs conserved among members of the same group are shown in blue. Examples of motifs conserved in only some members are shown in green. Non-homologous sequences between motifs are indicated by bars (image from Yanagisawa, 2002).

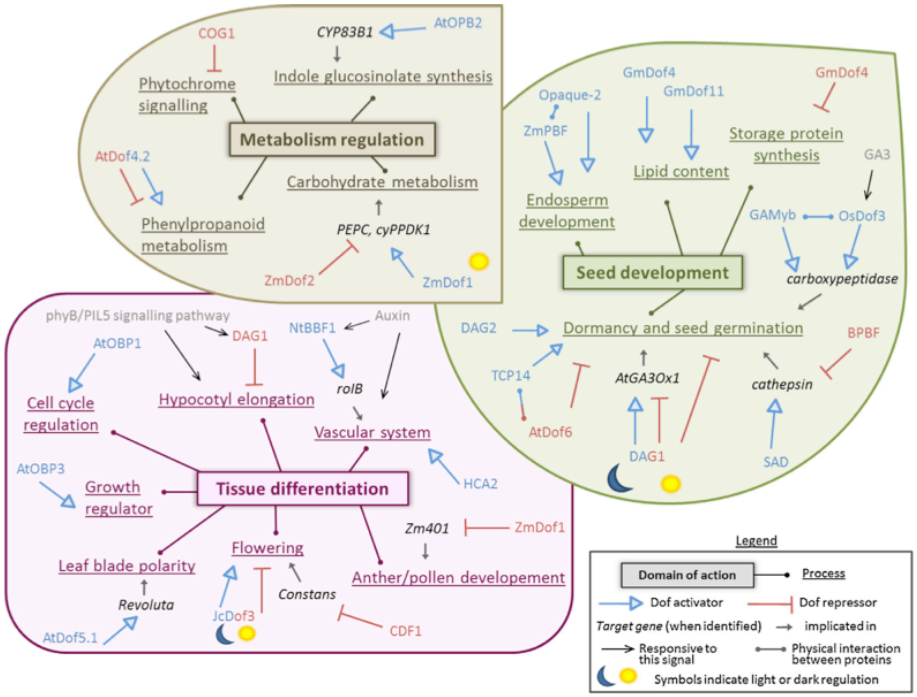

Figure 7 - *Examples of some processes which are known to involve regulation by DOF transcription factors* (images from Noguero et al., 2013).

Aims of the project

A Gene-Trap based Screen identifies a novel guard cell lineage regulator in *Arabidopsis thaliana*

The aim of my PhD is to isolate novel genes mediating plant growth. Growth is a key process in plant development and the result of a coordinated process of cell division and elongation. Progresses are being made in defining several signalling components underpinning the process of growth. Nonetheless, many aspects of growth control remain poorly understood. To identify novel gene involved in growth processes I screened a "Gene-Trap" (GT) collection of Arabidopsis. GUS staining reflects the expression profile of those genes where the GUS reporter gene is inserted. During the screen we have isolated a GUS reporter line with an insertion in a DOF–type transcription factor (TF, *AtDOF5.7*). These plants display GUS expression in guard cells, and young leaf primordia, which could suggest a role for *AtDOF5.7* in leaf growth and/or stomatal lineage specification or function. An independent loss-of-function, T-DNA–insertion allele of *AtDOF5.7* (dubbed *dof5.7-3*, No-0 ecotype) unexpectedly showed a shoot branching phenotype, characterised by reduced apical dominance and lack of axillary shoots outgrowth inhibition. The functional characterization of the role of *AtDOF5.7* in stomata development and shoot branching regulation led us to develop two parallel projects.

Part I - Role of *AtDOF5.7* in Guard Cell Development.

The differentiation of leaf epidermal cells into stomata is controlled by several well-characterized transcription factors that act sequentially to specify guard cell lineage initiation, progression and maturation (i.e. *SPCH*, *MUTE*, *FAMA*), however, how stomatal lineage is first specified and whether other factors play a role in its progression is poorly understood. In a recent publication, *AtDOF5.7* (in the paper and hereafter dubbed *STOMATAL CARPENTER 1*, *SCAP1*) has been shown to regulate the later stages of guard cell maturation and function. My initial results have suggested an early expression of *SCAP1* in leaf primordia before the appearance of GCs. The aim of this study is thus to define the role of *SCAP1* in the specification of guard cell lineage. To achieve this goal I generated independent *scap1* knock–down and gain of function alleles. Since the expression pattern of *SCAP1* overlaps with *SPCH,* a key regulator of GC lineage specification, transcript and confocal microscopy analyses have been performed to investigate variations in *SPCH* levels or activities in response to *SCAP1* perturbations.

Part III - Role of *AtDOF5.7* in shoot branching regulation

An independent *scap1* loss of function alleles, *scap1-3* (No-0 ecotype) produced visible branching alterations, which were not present in other backgrounds. Branching in Arabidopsis is regulated by several hormonal pathways including Strigolactones (SL), a new class of plant hormones, that inhibit growth of lateral buds. Mutants impaired in SL synthesis or signalling show a bushy phenotype, reminiscent of that of *scap1-3*. To elucidate if the shoot branching phenotype was caused by loss in SCAP1 activity independent *scap1* knock–down alleles in the No-0 background have been analysed. My results demonstrate the presence of a spontaneous mutation in the *scap1-3* genetic background causing the bushy phenotype (dubbed *more axillary meristems7, max7*). Independent experiments have been performed to test this hypothesis and to investigate the involvement of MAX7 in regulating SL accumulation and/or signalling.

Conclusions and future perspectives

DOF-type factors have been proposed to play an important role in GCs maturation and function based on an enrichment of DOF binding motifs in GC-specific genes (Cominelli et al., 2011; Galbiati et al., 2008; Plesch et al., 2001). Previously *SCAP1* was shown to be preferentially expressed in maturing GCs and to control GC morphology and movements (Negi et al., 2013). Here we report a new role for *SCAP1* in GC patterning. A detailed expression analysis throughout leaf development in *scap1-2* gene trap and *proSCAP1:GUS-GFP* transgenic plants have revealed two distinct *SCAP1* expression profiles (Figure 1, a-h - Part II). *SCAP1* expression was strong in maturing GCs, confirming previously observations (Figure 1, d,g,h and l - Part II) (Negi et al., 2013). However, *SCAP1* expression was also observed throughout emerging leaf primordia (Figure 1, a,b,e,f,i and j - Part II), when a leaf consists of few undifferentiated cells and no GCs are present (Figure 4, a – Part II). During leaf primordia development the *SCAP1* promoter is transcriptionally active in a region where stomata begin their differentiation (proximal to the stem) and much less active in the midvein region (Figure 1, b,c,f and g – Part II). This early *SCAP1* expression does not depend on *SPCH* since it persists in *spch* mutant plants (Figure 4, b – Part II). That *SCAP1* is not a direct target of SPCH is in agreement with a recently generated SPCH ChiP-seq dataset (Lau et al., 2014).

Functional studies implicate *SCAP1* in controlling cell divisions. *SCAP1* mutants display a significant decrease in GCs production and cell density compared with wild type (Figure 2, a,b and f; Figure S2, a – Part II). Conversely, *SCAP1* overexpression results in increased GCs production and cell density (Figure 2, d and f – Part II).

The analysis of plants carrying a *proSCAP1:GUS-GFP* fusion allowed me to conclude that the *SCAP1* promoter was active in both the epidermis and the mesophyll cells of leaves. However, this pattern does not clarify where SCAP1 protein acts to affect stomata development. To gain more insights in SCAP1 protein regulation I have generated transgenic plants carrying *SCAP1* fused to *YFP* under a constitutive promoter. Interestingly, despite the constitutive expression, SCAP1-YFP protein did not accumulate in all plant tissues. SCAP1-YFP was present in the mesophyll cells but was not detected in the epidermis although it was occasionally found in two neighbouring epidermal cells undergoing divisions (Figure 3 – Part II).

The above mentioned pattern of SCAP1 protein expression resembles the localization of SPCH. Also, the phenotype of *SCAP1* overexpressing plants is reminiscent of the phenotype of transgenic plants over expressing *SPCH* (Lampard et al., 2008; MacAlister et al., 2007). One hypothesis could be that *SCAP1* and *SPCH* may act together in protodermal cells to promote cell divisions and consequently stomatal cell linage initiation. To address this, we are designing co-localization experiments to confirm that SCAP1 and SPCH are expressed in the same cell type. Expression

studies in *SCAP1* overexpressing lines revealed that *SPCH* transcript is downregulated, which is in contrast with phenotypic result (Figure S3, a and b – Part II). In addition to transcriptional regulation, SPCH is regulated at the protein level. *SCAP1* may thus promote stomata production by enhancing SPCH protein activity. Indeed, evidence that SCAP1 rapidly promoted SPCH protein accumulation is provided in Figure 4 (Part II). Interestingly, we also detect a downregulation of *EPF2* transcript upon *SCAP1* overexpression, which could account for the observed increased SPCH accumulation (Figure S3, c and d – Part II).

My results highlight a previously unknown role for *SCAP1* in stomata development. *SCAP1* activation promotes accumulation of SPCH and downregulation of *EPF2*, a well-established negative regulator of SPCH protein stability. *SPCH*-expressing cells secrete EPF2 to inhibit stomatal lineage specification in neighbouring cells (Pillitteri & Dong, 2013). Moreover, *EPF2* promoter is directly targeted by SPCH (Lau et al., 2014) and *EPF2* expression in abolished in *spch* mutant plants (Hara et al., 2009). If present in the same cell type, *SCAP1* and *SPCH* may form a genetic loop to fine tune cell division potential of protodermal cells. A key control of this loop could involve a *SCAP1*-mediated downregulation of *EPF2* counteracting the previously proposed SPCH–mediated activation of *EPF2* (Lau et al., 2014). In a simple model, both SCAP1 and SPCH compete for the binding to the *EPF2* promoter (Figure 8 a). A Chromatin ImmunoPrecipitation (ChIP) assay could inform whether SCAP1 is able to bind to the *EPF2* promoter. DOF transcription factors are able to interact with other transcription factors on several promoter regions (Rueda-Romero et al., 2012; Wei et al., 2010; Yanagisawa, 1997; Yanagisawa & Schmidt, 1999). DOFs were shown to play an adjuvant role, enhancing the activation of transcription and facilitating the action of other transcription factors (Zhang et al., 1995). Examples of interactions between DOFs and bHLH transcription factors have been reported (H.-G. Kang, Foley, Oñate-Sánchez, Lin, & Singh, 2003). Because SPCH encodes a bHLH factor, SCAP1 might interact directly with SPCH sequestering it and preventing the activation of SPCH-specific target genes (Figure 8, b). For this purpose, it would be interesting to test whether SCAP1 can directly interact with SPCH in vivo and to answer this question we are designing a Biomolecular Fluorescence Complementation (BiFC) experiment.

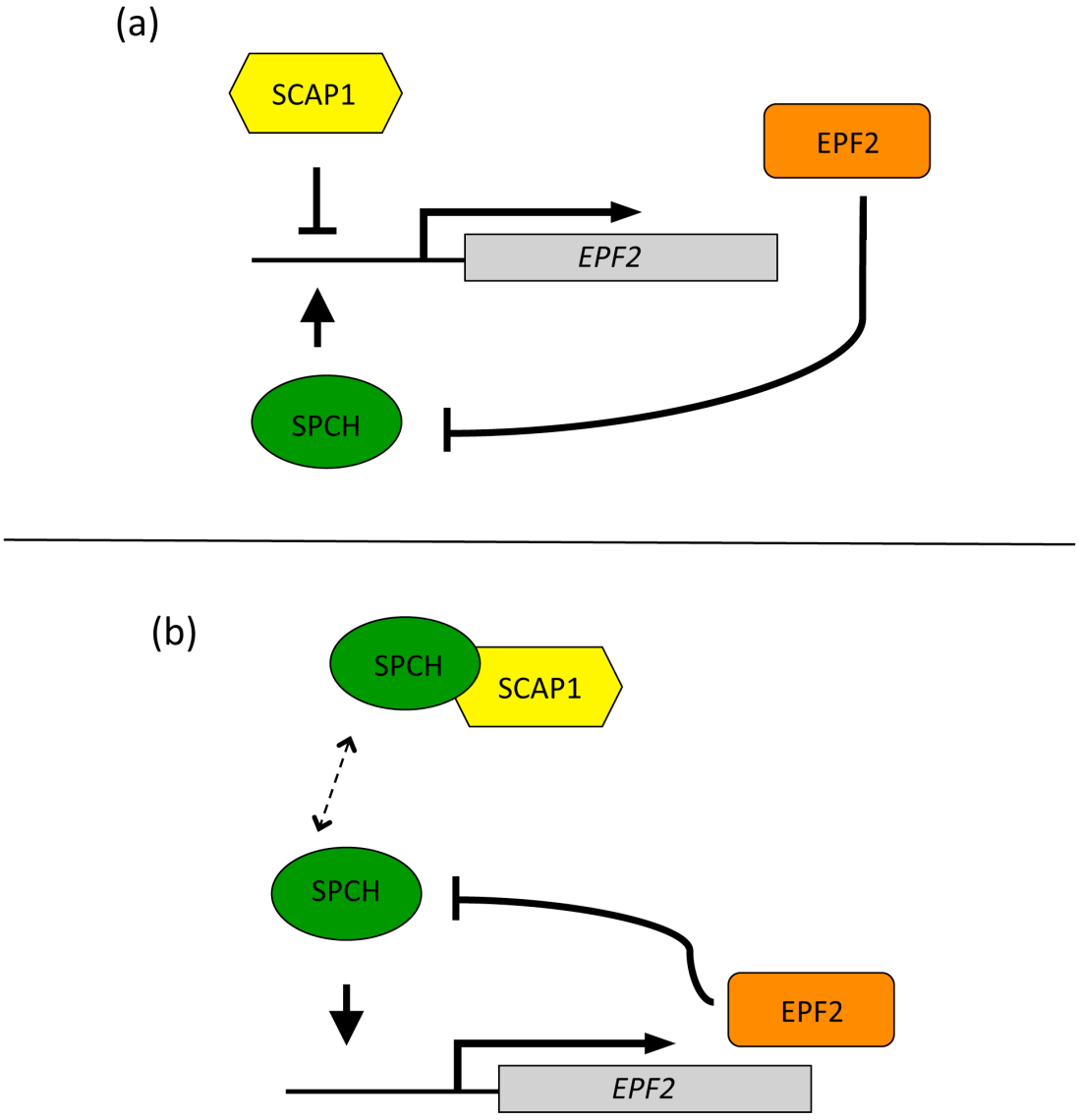

Figure 8 – *Two different models for SCAP1 action.*

References

Aoyama, T., & Chua, N. H. (1997). A glucocorticoid-mediated transcriptional induction system in transgenic plants. *The Plant Journal : for Cell and Molecular Biology*, *11*(3), 605–612.

Berger, D., & Altmann, T. (2000). A subtilisin-like serine protease involved in the regulation of stomatal density and distribution in Arabidopsis thaliana. *Genes & Development*, *14*(9), 1119–1131.

Bergmann, D. C., & Sack, F. D. (2007). Stomatal development. *Annual Review of Plant Biology*, *58*, 163–181.

Bergmann, D. C., Lukowitz, W., & Somerville, C. R. (2004). Stomatal development and pattern controlled by a MAPKK kinase. *Science (New York, N.Y.)*, *304*(5676), 1494–1497.

Boccalandro, H. E., Rugnone, M. L., Moreno, J. E., Ploschuk, E. L., Serna, L., Yanovsky, M. J., & Casal, J. J. (2009). Phytochrome B enhances photosynthesis at the expense of water-use efficiency in Arabidopsis. *Plant Physiology*, *150*(2), 1083–1092.

Casson, S. A., & Hetherington, A. M. (2010). Environmental regulation of stomatal development. *Current Opinion in Plant Biology*, *13*(1), 90–95.

Casson, S. A., Franklin, K. A., Gray, J. E., Grierson, C. S., Whitelam, G. C., & Hetherington, A. M. (2009). phytochrome B and PIF4 regulate stomatal development in response to light quantity. *Current Biology : CB*, *19*(3), 229–234.

Chen, H., Ahmad, M., Rim, Y., Lucas, W. J., & Kim, J.-Y. (2013). Evolutionary and molecular analysis of Dof transcription factors identified a conserved motif for intercellular protein trafficking. *The New Phytologist*, *198*(4), 1250–1260.

Cominelli, E., Galbiati, M., Albertini, A., Fornara, F., Conti, L., Coupland, G., & Tonelli, C. (2011). DOF-binding sites additively contribute to guard cell-specificity of AtMYB60 promoter. *BMC Plant Biology*, *11*, 162.

Cominelli, E., Galbiati, M., Vavasseur, A., Conti, L., Sala, T., Vuylsteke, M., et al. (2005). A guard-cell-specific MYB transcription factor regulates stomatal movements and plant drought tolerance. *Current Biology : CB*, *15*(13), 1196–1200.

Coupe, S. A., Palmer, B. G., Lake, J. A., Overy, S. A., Oxborough, K., Woodward, F. I., et al. (2006). Systemic signalling of environmental cues in Arabidopsis leaves. *Journal of Experimental Botany*, *57*(2), 329–341.

Dong, J., MacAlister, C. A., & Bergmann, D. C. (2009). BASL controls asymmetric cell division in Arabidopsis. *Cell*, *137*(7), 1320–1330.

Engineer, C. B., Ghassemian, M., Anderson, J. C., Peck, S. C., Hu, H., & Schroeder, J. I. (2014). Carbonic anhydrases, EPF2 and a novel protease mediate CO2 control of stomatal development. *Nature*, *513*(7517), 246–250.

Fornara, F., Panigrahi, K. C. S., Gissot, L., Sauerbrunn, N., RUhl, M., Jarillo, J. A., & Coupland, G. (2009). Arabidopsis DOF transcription factors act redundantly to reduce CONSTANS expression and are essential for a photoperiodic flowering response. *Developmental Cell*, *17*(1), 75–86.

Galbiati, M., Simoni, L., Pavesi, G., Cominelli, E., Francia, P., Vavasseur, A., et al. (2008). Gene trap lines identify Arabidopsis genes expressed in stomatal guard cells. *The Plant Journal : for Cell and Molecular Biology*, *53*(5), 750–762.

Geisler, M., Nadeau, J., & Sack, F. D. (2000). Oriented asymmetric divisions that generate the stomatal spacing pattern in arabidopsis are disrupted by the too many mouths mutation. *The Plant Cell Online*, *12*(11), 2075–2086.

Geisler, M., Yang, M., & Sack, F. D. (1998). Divergent regulation of stomatal initiation and patterning in organ and suborgan regions of the Arabidopsis mutants too many mouths and four lips. *Planta*, *205*(4), 522–530.

Gray, J. E., Holroyd, G. H., van der Lee, F. M., Bahrami, A. R., Sijmons, P. C., Woodward, F. I., et al. (2000). The HIC signalling pathway links CO2 perception to stomatal development. *Nature*, *408*(6813), 713–716.

Gualberti, G., Papi, M., Bellucci, L., Ricci, I., Bouchez, D., Camilleri, C., et al. (2002). Mutations in the Dof Zinc Finger Genes DAG2 and DAG1 Influence with Opposite Effects the Germination of Arabidopsis Seeds. *The Plant Cell Online*, *14*(6), 1253–1263.

Gudesblat, G. E., Schneider-Pizoń, J., Betti, C., Mayerhofer, J., Vanhoutte, I., van Dongen, W., et al. (2012). SPEECHLESS integrates brassinosteroid and stomata signalling pathways. *Nature Publishing Group*, *14*(5), 548–554.

Hara, K., KAJITA, R., Torii, K. U., Bergmann, D. C., & KAKIMOTO, T. (2007). The secretory peptide gene EPF1 enforces the stomatal one-cell-spacing rule. *Genes & Development*, *21*(14), 1720–1725.

Hara, K., Yokoo, T., KAJITA, R., Onishi, T., Yahata, S., Peterson, K. M., et al. (2009). Epidermal cell density is autoregulated via a secretory peptide, EPIDERMAL PATTERNING FACTOR 2 in Arabidopsis leaves. *Plant & Cell Physiology*, *50*(6), 1019–1031.

Hashimoto, M., Negi, J., Young, J., Israelsson, M., Schroeder, J. I., & Iba, K. (2006). Arabidopsis HT1 kinase controls stomatal movements in response to CO2. *Nature Cell Biology*, *8*(4), 391–397.

Heim, M. A., Jakoby, M., Werber, M., Martin, C., Weisshaar, B., & Bailey, P. C. (2003). The basic helix-loop-helix transcription factor family in plants: a genome-wide study of protein structure and functional diversity. *Molecular Biology and Evolution*, *20*(5), 735–747.

Hetherington, A. M., & Woodward, F. I. (2003). The role of stomata in sensing and driving environmental change. *Nature*, *424*(6951), 901–908.

Hu, H., Boisson-Dernier, A., Israelsson-Nordström, M., Böhmer, M., Xue, S., Ries, A., et al. (2010). Carbonic anhydrases are upstream regulators of CO2-controlled stomatal movements in guard cells. *Nature Publishing Group*, *12*(1), 87–93– sup pp 1–18.

Hunt, L., & Gray, J. E. (2009). The signaling peptide EPF2 controls asymmetric cell divisions during stomatal development. *Current Biology : CB*, *19*(10), 864–869.

Hunt, L., Bailey, K. J., & Gray, J. E. (2010). The signalling peptide EPFL9 is a positive regulator of stomatal development. *The New Phytologist*, *186*(3), 609–614.

Jewaria, P. K., Hara, T., Tanaka, H., KONDO, T., Betsuyaku, S., Sawa, S., et al. (2013). Differential effects of the peptides Stomagen, EPF1 and EPF2 on activation of MAP kinase MPK6 and the SPCH protein level. *Plant & Cell Physiology*, *54*(8), 1253–1262.

Kanaoka, M. M., Pillitteri, L. J., Fujii, H., Yoshida, Y., Bogenschutz, N. L., Takabayashi, J., et al. (2008). SCREAM/ICE1 and SCREAM2 specify three cell-state transitional steps leading to arabidopsis stomatal differentiation. *The Plant Cell Online*, *20*(7), 1775–1785.

Kang, C.-Y., Lian, H.-L., Wang, F.-F., Huang, J.-R., & Yang, H.-Q. (2009). Cryptochromes, phytochromes, and COP1 regulate light-controlled stomatal development in Arabidopsis. *The Plant Cell*, *21*(9), 2624–2641.

Kang, H.-G., Foley, R. C., Oñate-Sánchez, L., Lin, C., & Singh, K. B. (2003). Target genes for OBP3, a Dof transcription factor, include novel basic helix-loop-helix domain proteins inducible by salicylic acid. *The Plant Journal*, *35*(3), 362–372.

Khan, M., Rozhon, W., Bigeard, J., Pflieger, D., Husar, S., Pitzschke, A., et al. (2013). Brassinosteroid-regulated GSK3/Shaggy-like Kinases Phosphorylate Mitogen-activated Protein (MAP) Kinase Kinases, Which Control Stomata Development in Arabidopsis thaliana. *Journal of Biological Chemistry*, *288*(11), 7519–7527.

Kim, H.-S., Kim, S. J., Abbasi, N., Bressan, R. A., Yun, D.-J., Yoo, S.-D., et al. (2010). The DOF transcription factor Dof5.1 influences leaf axial patterning by promoting Revoluta transcription in Arabidopsis. *The Plant Journal : for Cell and Molecular Biology*, *64*(3), 524–535.

Kim, T.-W., Michniewicz, M., Bergmann, D. C., & Wang, Z.-Y. (2012). Brassinosteroid regulates stomatal development by GSK3-mediated inhibition of a MAPK pathway. *Nature*, *482*(7385), 419–422.

Kisu, Y., Ono, T., Shimofurutani, N., Suzuki, M., & Esaka, M. (1998). Characterization and expression of a new class of zinc finger protein that binds to silencer region of ascorbate oxidase gene. *Plant & Cell Physiology*, *39*(10), 1054–1064.

KONDO, T., KAJITA, R., Miyazaki, A., Hokoyama, M., Nakamura-Miura, T., Mizuno, S., et al. (2010). Stomatal density is controlled by a mesophyll-derived signaling molecule. *Plant & Cell Physiology*, *51*(1), 1–8.

Krebs, J., Mueller-Roeber, B., & Ruzicic, S. (2010). A novel bipartite nuclear localization signal with an atypically long linker in DOF transcription factors. *Journal of Plant Physiology*, *167*(7), 583–586.

Lake, J. A., Quick, W. P., Beerling, D. J., & Woodward, F. I. (2001). Plant development: signals from mature to new leaves. *Nature*, *411*(6834), 154–154.

Lake, J. A., Woodward, F. I., & Quick, W. P. (2002). Long-distance CO2 signalling in plants. *Journal of Experimental Botany*, *53*(367), 183–193.

Lampard, G. R., Lukowitz, W., Ellis, B. E., & Bergmann, D. C. (2009). Novel and expanded roles for MAPK signaling in Arabidopsis stomatal cell fate revealed by cell type-specific manipulations. *The Plant Cell*, *21*(11), 3506–3517.

Lampard, G. R., MacAlister, C. A., & Bergmann, D. C. (2008). Arabidopsis stomatal initiation is controlled by MAPK-mediated regulation of the bHLH SPEECHLESS. *Science (New York, N.Y.)*, *322*(5904), 1113–1116.

Lau, O. S., Davies, K. A., Chang, J., Adrian, J., Rowe, M. H., Ballenger, C. E., & Bergmann, D. C. (2014). Direct roles of SPEECHLESS in the specification of stomatal self-renewing cells. *Science (New York, N.Y.)*, *345*(6204), 1605–1609.

Lee, J. S., Kuroha, T., Hnilova, M., Khatayevich, D., Kanaoka, M. M., McAbee, J. M., et al. (2012). Direct interaction of ligand-receptor pairs specifying stomatal patterning. *Genes & Development*, *26*(2), 126–136.

Lee, J.-Y., Colinas, J., Wang, J. Y., Mace, D., Ohler, U., & Benfey, P. N. (2006). Transcriptional and posttranscriptional regulation of transcription factor expression in Arabidopsis roots. *Proceedings of the National Academy of Sciences*, *103*(15), 6055–6060.

MacAlister, C. A., & Bergmann, D. C. (2011). Sequence and function of basic helix-loop-helix proteins required for stomatal development in Arabidopsis are deeply conserved in land plants. *Evolution & Development*, *13*(2), 182–192.

MacAlister, C. A., Ohashi-Ito, K., & Bergmann, D. C. (2007). Transcription factor control of asymmetric cell divisions that establish the stomatal lineage. *Nature*, *445*(7127), 537–540.

Masle, J., Gilmore, S. R., & Farquhar, G. D. (2005). The ERECTA gene regulates plant transpiration efficiency in Arabidopsis. *Nature*, *436*(7052), 866–870.

Mora-García, S., Vert, G., Yin, Y., Caño-Delgado, A., Cheong, H., & Chory, J. (2004). Nuclear protein phosphatases with Kelch-repeat domains modulate the response to brassinosteroids in Arabidopsis. *Genes & Development*, *18*(4), 448–460.

Nadeau, J. A. (2009). Stomatal development: new signals and fate determinants. *Current Opinion in Plant Biology*, *12*(1), 29–35.

Nadeau, J. A., & Sack, F. D. (2002). Control of stomatal distribution on the Arabidopsis leaf surface. *Science (New York, N.Y.)*, *296*(5573), 1697–1700.

Nadeau, J. A., & Sack, F. D. (2003). Stomatal development: cross talk puts mouths in place. *Trends in Plant Science*, *8*(6), 294–299.

Negi, J., Moriwaki, K., Konishi, M., Yokoyama, R., Nakano, T., Kusumi, K., et al. (2013). A Dof Transcription Factor, SCAP1, Is Essential for the Development of Functional Stomata in Arabidopsis. *Current Biology : CB*, *23*(6), 479–484.

Noguero, M., Atif, R. M., Ochatt, S., & Thompson, R. D. (2013). The role of the DNA-binding One Zinc Finger (DOF) transcription factor family in plants. *Plant Science : an International Journal of Experimental Plant Biology*, *209*, 32–45.

Ohashi-Ito, K., & Bergmann, D. C. (2006). Arabidopsis FAMA controls the final proliferation/differentiation switch during stomatal development. *The Plant Cell*, *18*(10), 2493–2505.

Ohki, S., Takeuchi, M., & Mori, M. (2011). The NMR structure of stomagen reveals the basis of stomatal density regulation by plant peptide hormones. *Nature Communications*, *2*, 512.

Papi, M., Sabatini, S., Bouchez, D., Camilleri, C., Costantino, P., & Vittorioso, P. (2000). Identification and disruption of an Arabidopsis zinc finger gene controlling seed germination. *Genes & Development*, *14*(1), 28–33.

Pillitteri, L. J., & Dong, J. (2013). Stomatal development in Arabidopsis. *The Arabidopsis Book / American Society of Plant Biologists*, *11*, e0162.

Pillitteri, L. J., & Torii, K. U. (2012). Mechanisms of stomatal development. *Annual Review of Plant Biology*, *63*, 591–614.

Pillitteri, L. J., Bogenschutz, N. L., & Torii, K. U. (2008). The bHLH protein, MUTE, controls differentiation of stomata and the hydathode pore in Arabidopsis. *Plant & Cell Physiology*, *49*(6), 934–943.

Pillitteri, L. J., Sloan, D. B., Bogenschutz, N. L., & Torii, K. U. (2007). Termination of asymmetric cell division and differentiation of stomata. *Nature*, *445*(7127), 501–505.

Pires, N., & Dolan, L. (2010). Origin and diversification of basic-helix-loop-helix proteins in plants. *Molecular Biology and Evolution*, *27*(4), 862–874.

Plesch, G., Ehrhardt, T., & Mueller-Roeber, B. (2001). Involvement of TAAAG elements suggests a role for Dof transcription factors in guard cell-specific gene expression. *The Plant Journal*, *28*(4), 455–464.

Riechmann, J. L., Heard, J., Martin, G., Reuber, L., Keddie, J., Adam, L., et al. (2000). Arabidopsis transcription factors: genome-wide comparative analysis among eukaryotes. *Science (New York, N.Y.)*, *290*(5499), 2105–2110.

Robinson, S., Barbier de Reuille, P., Chan, J., Bergmann, D., Prusinkiewicz, P., & Coen, E. (2011). Generation of spatial patterns through cell polarity switching. *Science (New York, N.Y.)*, *333*(6048), 1436–1440.

Rueda-Romero, P., Barrero-Sicilia, C., Gómez-Cadenas, A., Carbonero, P., & Oñate-Sánchez, L. (2012). Arabidopsis thaliana DOF6 negatively affects germination in non-after-ripened seeds and interacts with TCP14. *Journal of Experimental Botany*, *63*(5), 1937–1949.

Sessions, A., Nemhauser, J. L., McColl, A., Roe, J. L., Feldmann, K. A., & Zambryski, P. C. (1997). ETTIN patterns the Arabidopsis floral meristem and reproductive organs. *Development*, *124*(22), 4481–4491.

Shpak, E. D., McAbee, J. M., Pillitteri, L. J., & Torii, K. U. (2005). Stomatal patterning and differentiation by synergistic interactions of receptor kinases. *Science (New York, N.Y.)*, *309*(5732), 290–293.

Skirycz, A., Radziejwoski, A., Busch, W., Hannah, M. A., Czeszejko, J., Kwaśniewski, M., et al. (2008). The DOF transcription factor OBP1 is involved in cell cycle regulation in Arabidopsis thaliana. *The Plant Journal : for Cell and Molecular Biology*, *56*(5), 779–792.

Sugano, S. S., Shimada, T., Imai, Y., Okawa, K., Tamai, A., Mori, M., & Hara-Nishimura, I. (2010). Stomagen positively regulates stomatal density in Arabidopsis. *Nature*, *463*(7278), 241–244.

Tanaka, Y., Nose, T., Jikumaru, Y., and Kamiya, Y. (2013). ABA inhibits entry into stomatal lineage development in Arabidopsis leaves. *Plant J*, 74, 448-457.

Wei, P.-C., Tan, F., Gao, X.-Q., Zhang, X.-Q., Wang, G.-Q., Xu, H., et al. (2010). Overexpression of AtDOF4.7, an Arabidopsis DOF family transcription factor, induces floral organ abscission deficiency in Arabidopsis. *Plant Physiology*, *153*(3), 1031–1045.

Woodward, F. I. (1987). Stomatal numbers are sensitive to increases in CO2 from pre-industrial levels. *Nature*, *327*(6123), 617–618.

Yanagisawa, S. (1997). Dof DNA-binding domains of plant transcription factors contribute to multiple protein-protein interactions. *European Journal of Biochemistry / FEBS*, *250*(2), 403–410.

Yanagisawa, S. (2001). The transcriptional activation domain of the plant-specific Dof1 factor functions in plant, animal, and yeast cells. *Plant & Cell Physiology*, *42*(8), 813–822.

Yanagisawa, S. (2002). The Dof family of plant transcription factors. *Trends in Plant Science*, *7*(12), 555–560.

Yanagisawa, S., & Schmidt, R. J. (1999). Diversity and similarity among recognition sequences of Dof transcription factors. *The Plant Journal : for Cell and Molecular Biology*, *17*(2), 209–214.

Yang, H. Q., Tang, R. H., & Cashmore, A. R. (2001). The signaling mechanism of Arabidopsis CRY1 involves direct interaction with COP1. *The Plant Cell Online*, *13*(12), 2573–2587.

Yoo, C. Y., Hasegawa, P. M., & Mickelbart, M. V. (2011). Regulation of stomatal density by the GTL1 transcription factor for improving water use efficiency. *Plant Signaling & Behavior*, *6*(7), 1069. doi:10.4161/psb.6.7.15254

Zhang B., Chen W., Foley R.C., Buttner M., Singh K.B. (1995). Interactions between dis- tinct types of DNA binding proteins enhance binding to ocs element promoter sequences, *Plant Cell,* 7, 2241–2252.

Zhao, L., & Sack, F. D. (1999). Ultrastructure of stomatal development in Arabidopsis (Brassicaceae) leaves. *American Journal of Botany*, *86*(7), 929–939.