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A brief history of the TDIF-PXY signalling module: Balancing meristem identity and differentiation during vascular development.

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Abstract

A significant proportion of terrestrial biomass is constituted of xylem cells that make up woody plant tissue. Xylem is required for water transport, and is present in the vascular tissue with a second conductive tissue, phloem, required primarily for nutrient transport. Both xylem and phloem are derived from cell divisions in vascular meristems known as the cambium and procambium. One major component that influences several aspects of plant vascular development, including cell division in the vascular meristem, vascular organisation and differentiation of vascular cell types, is a signalling module characterised by a peptide ligand called TDIF and its cognate receptor, PXY. In this review, we explore the literature that describes signalling components, phytohormones and transcription factors that interact with these two central factors, to control the varying outputs required in vascular tissues for normal organisation and elaboration of plant vascular tissue.

Introduction

In higher plants, post-embryonic tissues are derived from pluripotent stem cell populations present in niches referred to as meristems. Stem cell niches are composed of central organizing cells with adjacent dividing stem cells that provide cells for new organs. Meristems that are responsible for length-wise plant growth are located at the root and shoot apices. Radial expansion also occurs via divisions in meristematic tissues, referred to as the cambium and procambium, which are present in the vascular tissue. Vascular tissue supports length-wise growth by providing both physical support and long-distance fluid and nutrient transport. It is constituted of two major transport tissues; the phloem, which is primarily responsible for movement of photosynthate from the leaves to the root system, and the xylem, which is responsible for the movement of water and solutes from the root to the shoot. The xylem is also responsible for the majority of the plants mechanical strength, due to the presence of cells with large woody secondary cell walls. The cambium and procambium are positioned between xylem and phloem, and cells within these meristematic tissues divide down their long axis in a highly oriented manner, perpendicular to the radial axis of the stem. These divisions within the cambium displace older cells to the periphery of the meristem where they take on either xylem identity towards the centre of the stem, or a phloem identity towards the outside. Vascular meristems are responsible for generating the majority of plant biomass, which is mostly constituted of the xylem. This woody tissue represents a huge renewable resource of energy and biomaterials.

An increasing number of molecular and genetic components that include phytohormones, components of signalling mechanisms, and transcription factors have been identified and characterised that are required to initiate, pattern and expand the vascular tissue. These components have been summarised in a number recent reviews (Lucas *et al.*, 2013; Furuta *et al.*, 2014; Ohashi-Ito & Fukuda, 2014; Jouannet *et al.*, 2015). One such mechanism is characterised by the peptide ligand TDIF (TRACHEARY ELEMENT DIFFERENTIATION INHIBITORY FACTOR) and its cognate receptor PHLOEM INTERCALATED WITH XYLEM (PXY). The interaction of these factors results in particularly complex outputs. This ligand-receptor pair is thought to regulate the rate of cell division in the cambium and procambium, exclude xylem identity from the division zone and also control vascular organisation via interactions with a number of other signalling components and transcriptional regulators (Ito *et al.*, 2006; Fisher & Turner, 2007; Hirakawa *et al.*, 2008; Etchells & Turner, 2010b). Here we focus on aspects of vascular development influenced by TDIF/PXY signalling that describes how the complex outputs of this signalling pathway are coordinated to control vascular development.

Peptides that regulate stem cell fate

Transdifferentiation assays have proved a powerful tool for identification of factors influence vascular development (Fukuda, 1997; Demura *et al.*, 2002; Kubo *et al.*, 2005). These assays make use of mechanically isolated leaf mesophyll cells that are subjected to auxin and cytokinin treatments enabling them to dedifferentiate into procambium-like cells and subsequently take on a tracheary element identity reminiscent of mature xylem in the absence of cell division (Fukuda & Komamine, 1980). TDIF was first identified as a dodecapeptide (His-Glu-Val-Hyp-Ser-Gly-Hyp-Asn-Pro-Ile-Ser-Asn) that promoted both cell division and prevented assumption of tracheary element identity in this system. The *CLAVATA3*/*EMBRYO SURROUNDING REGION* (*CLE*) gene family encodes for ligands which interact with receptor-like kinase receptors present at the cell surface. The mature TDIF peptide is derived independently from two *CLE* family members, *CLE41* and *CLE44*, that are translated as 100 amino acid proteins which are cleaved and the proline residues hydroxylated to form the mature peptide (Ito *et al.*, 2006). A closely related gene, *CLE42*, encodes a dodecapeptide with similar activity (Etchells & Turner, 2010b) but differs at by a single amino acid (Glu → Gly at the second amino acid of the mature peptide) (Ito *et al.*, 2006).

In Arabidopsis there are 32 *CLE* family genes (Oelkers *et al.*, 2008) which have been placed in putative functional groups based on phenotypes of constitutive overexpression lines. Plants

overexpressing the *CLE* genes from which TDIF is derived demonstrated a loss of apical dominance (Strabala *et al.*, 2006). This phenotype is fairly common in plants that have either xylem differentiation defects, such as *irregular xylem3* (*irx3*) mutants, which lack large secondary cell walls, and/or in procambium over-proliferation mutants, such as *acaulis5* (*acl5*) and *high cambial activity* (*hca*) (Taylor *et al.*, 1999; Hanzawa *et al.*, 2000; Pineau *et al.*, 2005). Consistent with a loss of apical dominance, considerable increases in cell numbers in the vascular tissue of *CLE41*, *CLE42* and *CLE44* over-expression lines were subsequently observed (Figure 1A-B) which were accompanied by increases in the expression of genes that mark dividing cells (Hirakawa *et al.*, 2008; Whitford *et al.*, 2008; Etchells & Turner, 2010b). Consequently, observations in both the transdifferentiation assay and *in planta* suggested that TDIF promotes cell division in vascular tissue.

A receptor for TDIF ligand

Stem cell populations in the shoot and root have been shown to be under strict control to limit their size in order to maintain the balance between cell division and differentiation. Critical in this regulation are peptides from the CLE/ESR family (Fletcher *et al.*, 1999; Schoof *et al.*, 2000; Stahl *et al.*, 2009). In the shoot apical meristem, CLV3 peptide controls meristem size via binding to the LRR-RLK receptor CLAVATA1 (CLV1) (Kondo *et al.*, 2006; Ogawa *et al.*, 2008), and as such cell-surface receptors related to CLV1 are prime candidates for receptors to other CLE peptides. Seedlings challenged with TDIF treatment in liquid culture exhibit a striking reduction in xylem cell differentiation, consistent with the original observations made in the transdifferentiation assay. Using this knowledge, a collection of LRR-RLK receptor mutants were treated with TDIF in order to identify mutants that failed to respond (Hirakawa *et al.*, 2008). Lines carrying a T-DNA insertion in a receptor kinase gene, present in a clade adjacent to that containing *CLV1* and closely related *BARELY ANY MERISTEM* (*BAM*) genes (Shiu & Bleecker, 2001; DeYoung *et al.*, 2006) failed to respond to TDIF treatment. Photoaffinity labelling demonstrated that TDIF bound to this receptor which was named TDR (for TDIF Receptor) (Hirakawa *et al.*, 2008). *TDR* had been previously described as being encoded by the *PHLOEM INTERACTED WITH XYLEM* (*PXY*) gene, required for normal organisation of vascular tissue (Fisher & Turner, 2007). In wild type plants vascular tissue is ordered xylem, procambium, phloem in distinct domains along the radial axis of the stem (Figure 1A). In *pxy* mutants, phloem and xylem lacked spatial separation, with phloem intercalated with xylem (Figure 1C). In initiating vascular tissue, cell divisions that in wild type formed perpendicular to the radial axis of the stem were observed

at altered orientations. Changes were also observed in the xylem. Wild type xylem cells are characteristically long and straight and run parallel to the apical – basal axis of the stem. In *pxy* mutants the xylem cells were curved, running in and out of the plane of longitudinal sections (Etchells & Turner, 2010a). Arabidopsis xylem is mostly constituted of three cell types; vessels, parenchyma and fibres. Vessels are large water conducting cells with pitted secondary cell walls. Smaller parenchyma and fibre cells fill the gaps between vessels. In established vascular tissue, fibres, which develop secondary walls are prevalent, but in young vascular tissue it is typically parenchyma, which do not have secondary cell walls, that are formed. While the number of differentiated cells present in the xylem was reported as unchanged in vascular bundles of 6 week old *pxy* mutants, the ratio of vessels to other xylem cell types was altered, with *pxy* demonstrating a reduction in vessel number (Fisher & Turner, 2007). In 4 week old inflorescence stems, phloroglucinol (which marks the lignin component in xylem secondary cell walls) appears in ectopic positions in *pxy* mutant vascular bundles, consistent with observations made in the transdifferentiation assay, suggesting that TDIF-PXY represses xylem differentiation (Figure 2).

The loss of vascular organisation in *pxy* mutants, increases in cell division in the procambium of *CLE41* and *CLE42* overexpression lines, and changes to xylem differentiation in both cell culture and plant lines led to hypotheses suggesting that TDIF and PXY represent a multifunctional signalling module with complex outputs. Overexpression phenotypes of *CLE41* and *CLE42* are entirely suppressed by *pxy*, suggesting that all known outputs of TDIF signalling are via the PXY receptor (Etchells & Turner, 2010b). It is unknown how or if these outputs, i.e. control of cell division; vascular organisation; and xylem differentiation, are inter-related and/or integrated. However, some understanding of the differing outputs, discussed below, has followed identification of transcription factor targets of TDIF-PXY, signal components that act downstream of PXY in the cytoplasm, and other signalling pathways that cross-talk with TDIF-PXY.

TDIF, PXY and promotion of vascular cell division.

As with the identification of the TDIF receptor, identification of the first transcriptional target of PXY signalling demonstrated homology with signalling systems in shoot and root apical meristems. In the shoot, the expression of the *WUSCHEL* (*WUS*) transcription factor is regulated via the interaction between CLV3 peptide and CLV1 receptor (Brand *et al.*, 2000; Schoof *et al.*, 2000). Furthermore, in roots, expression of the *WUSCHEL-related HOMEOBOX 5* (*WOX5*) transcription factor responds to changes in CLE40 levels (Stahl *et*

al., 2009), through the LRR-RLK ARABIDOPSIS CRINKLY 4 (ACR4) receptor (Stahl *et al.*, 2009). It therefore followed that *WOX* genes may be targets of TDIF-PXY signalling. Rapid induction of *WOX4* expression was observed following incubation of wild type seedlings in liquid culture with TDIF (<30 mins) but not in *pxy* mutants, demonstrating that TDIF controls *WOX4* expression through the PXY receptor and thus identifying *WOX4* as the first transcriptional target of TDIF-PXY (Hirakawa *et al.*, 2010). When wild type plants were subjected to TDIF treatment for seven days, increases in the number of procambium cells were observed that were not present in *wox4* lines under similar conditions. Genetic analysis of *wox4* mutants also suggested that, while procambium cell number was reduced, xylem differentiation appeared unaffected. Consequently, *WOX4* is thought to be involved in the regulation of cellular proliferation but not xylem differentiation, suggesting that these two TDIF-PXY outputs are genetically separable (Hirakawa *et al.*, 2010). Evidence suggests that WOX4 acts downstream of PXY signalling to promote vascular cell division in conjunction with a second WOX transcription factor, *WOX14*, the expression of which is increased in response to longer treatment with TDIF as *wox14* enhances the cell division defect of *wox4* mutants (Etchells *et al.*, 2013).

Repression of xylem differentiation by PXY signalling.

While identification of TDIF-PXY transcription factor targets led to some understanding of cell division outputs of the TDIF-PXY signalling module, factors that interact with the PXY cytoplasmic domain have been identified as mediating control of xylem differentiation. In plants, LRR RLK signalling on the cytoplasmic side of the cell membrane is mediated by either mitogen activated protein kinases, glycogen synthase kinase 3 proteins (GSK3's), or in some cases, combinations of the two (Xu & Zhang, 2015; Youn & Kim, 2015). Related GSK3 proteins BRASSINOSTEROID INSENSITIVE 2 (BIN2), BIN2-LIKE 1 (BIL1), BIL2, SHAGGY-RELATED KINASE 11 (ATSK11) and ATSK13 were found to interact with PXY in yeast two-hybrid assays, and these interactions were confirmed *in planta* by fluorescence resonance energy transfer. In tobacco transient assays, the interaction between GSK3's and PXY was shown to occur at the plasma membrane, and only in the presence of TDIF. Consequently, the perception of TDIF by PXY results in promotion of GSK3 activity (Kondo *et al.*, 2014). The interaction between GSK3's and PXY is thought to specify repression of xylem identity. GSK3 mutant/knockdown lines which were subjected to an 11 day treatment of TDIF in liquid culture demonstrated xylem differentiation in areas where mature xylem was not observed in wild type plants subjected to the same treatment. In

contrast, the rate of cell division in wild type and GSK3 mutants was unchanged. This indicates that while the repression of xylem identity output of PXY signalling is via a PXY - GSK3 interaction, PXY influence on vascular cell division is independent of GSK3's (Kondo *et al.*, 2014), and presumably mediated by as yet unknown signalling components.

The interaction between PXY and GSK3's is thought to result in negative regulation of transcription factors BRASSINAZOLE-RESISTANT 1 (BZR1) and BRI1-EMS-SUPPRESSOR 1 (BES1) by phosphorylation (Figure 3). Dominant mutations in genes encoding these transcriptional regulators, *bes-1d* and *bzr1-d* result in proteins not subject to this phosphorylation-mediated repression (Yin *et al.*, 2002; Yin *et al.*, 2005). *bes-1d*, but not *bzr-1d* lines, were reported to demonstrate a reduction in the number of procambium cells. This suggests that cells normally present in the procambium were subject to premature differentiation into xylem, consistent with a model by which BES1 promotes xylem identity, and is prevented from doing so in the procambium via negative regulation by interactions between TDIF, PXY and GSK3's (Kondo *et al.*, 2014).

In contrast to TDIF-PXY, brassinosteroid signalling has been reported to promote xylem identity (Yamamoto *et al.*, 2001; Yamamoto *et al.*, 2007). Brassinosteroid receptor genes *BRASSINOSTEROID INSENSITIVE* (*BRI*), *BRI-LIKE1* (*BRL1*), and *BRL2* are expressed broadly in the vascular tissue (Cano-Delgado *et al.*, 2004). Signalling through the BRI(L) receptors, negatively regulates the GSK3 BIN2 (Li & Nam, 2002). Negative regulation of BIN2 leads to hypophosphorylation of BZR1 and BES1 transcription factors and therefore activation (and nuclear localisation) (Yin *et al.*, 2002; Yin *et al.*, 2005). A balance between TDIF-PXY and brassinosteroid through BRI receptors likely maintains the balance between proliferation in the procambium and differentiation in the xylem via opposing regulation of GSK3's and their targets (Figure 3).

Vascular organisation defects in plants with altered TDIF-PXY signalling: Cause or effect?

The spatial separation of ligand and receptor can allow for restriction of stem cell identity to a limited number of cells. PXY-TDIF is a good example of this as the PXY transcript is procambium, i.e. stem cell expressed, while CLE41 and CLE44 transcript expression is restricted to the phloem. This suggests that the CLE41/44 protein likely moves a short distance and that this movement may set up the spatial vascular pattern and potentially, stem cell maintenance. The original model for PXY control of vascular organisation (Fisher & Turner, 2007) drew on a mechanism that controls the highly oriented division of cell

divisions during *C. elegans* embryogenesis. The cell lineage of *C. elegans* is almost entirely invariant from one individual to the next. The *C. elegans* zygote undergoes asymmetric cell divisions to produce a series of founder cells and stem cells responsible for all cells in the organism. Specifically, the P1 cell divides transversely to produce a founder cell (EMS) and a posterior stem cell (P2). The EMS cell then divides to produce the E cell and the MS cell. In this system, the cell division plane of the EMS cell is defined by the position of the adjacent P2 cell. P2 confers the positional information to the dividing EMS cell via a ligand that controls the WNT signalling pathway in the EMS. Consequently the orientation of the cell division can be manipulated by altering the position of the P2 cell (Goldstein, 1995; Schlesinger *et al.*, 1999). If PXY acts in a similar manner and interprets positional information derived from TDIF, in a background such as *35S::CLE41* where TDIF is derived from all cells and not just those of the phloem, changes in vascular organisation should be evident. This is the phenotype that is observed in *35S::CLE41* lines which are characterised by intercalation of xylem and phloem and changes to the orientation of cell division in the procambium (Figure 1A-B). Furthermore, such disrupted vascular organisation is unlikely to be explained simply as a response to increased ligand because when *CLE41* is over-expressed in a phloem-specific manner such as in *SUC2::CLE41* lines, plants retain wild type-like vascular organisation (Etchells & Turner, 2010b).

Alternative explanations for loss of vascular organisation in *pxy* mutants have been suggested by comparison of *pxy* mutant phenotypes to those of plants demonstrating defects in the shoot apical meristem. One of the hallmarks of mutants with a defective shoot apical meristem, is meristem re-initiation at positions adjacent to the (now) differentiated tissue where the meristem forms in wild type. For example, the shoot meristem is consumed during embryogenesis in the *shootmeristemless* mutant, but frequently, an ectopic meristem will form at the hypocotyl (Barton & Poethig, 1993). Similarly, *pinhead* mutants typically develop a lateral organ in the place where the meristem forms in wild type, but in the majority of cases, a new meristem is formed at the axil of the ectopic lateral organ (McConnell & Barton, 1995). Finally, in the absence of *WUSCHEL*, which encodes a relative of *WOX4*, meristems are repeatedly consumed and reinitiated (Laux *et al.*, 1996). Consequently, with respect to these mutant analyses, a hypothesis was put forward referred to as the cavity model suggesting that the loss of PXY results in a cavity, i.e. consumption of the vascular meristem in some cell files. Loss of procambium cells in some files would result in adjacent xylem and phloem. Subsequent procambium re-initiation in a manner similar to that observed in apical meristem mutants would lead to a further disruption of pattern and

intercalation of xylem and phloem (Hirakawa *et al.*, 2010). This hypothesis was supported by the phenotype of *pxy wox4* lines that demonstrate vasculature with greater levels of disorganisation than that of *pxy* single mutants. In contrast, other observations in different genetic backgrounds argue against this hypothesis. *wox4* single mutants demonstrate reductions in vascular proliferation in line with those observed in *pxy* single mutants however, no loss of vascular organisation is apparent (Hirakawa *et al.*, 2010; Etchells *et al.*, 2013). As the activity of the vascular meristem is similar in both cases, according to the cavity model, one would expect a loss of organisation in *wox4* mutants, however, *wox4* lines lack any organisation defects. This suggests that vascular organisation and vascular proliferation are genetically separable. Genetic separation of vascular organisation and cell division is supported by observations of *pxy* mutants in genetic backgrounds that lack a functional copy of the *ERECTA* (*ER*) receptor like kinase. ER regulates a number of processes in plant development including development of stomata (Shpak *et al.*, 2005), and regulation of cell division - a function which it carries out redundantly with related receptor kinases, *ERECTA-like 1* (*ERL1*) and *ERL2* (Shpak *et al.*, 2004). *ER* is expressed in the phloem (Uchida & Tasaka, 2013), so although expression does not overlap with that of procambium-expressed *PXY*, *er pxy* mutants demonstrate considerable increases in vascular organisation defects (Figure 4). Importantly, these mutants display no vascular proliferation defects as *pxy er* vascular bundles have the same numbers of cells of those of *pxy* single mutant plants, again, pointing to genetically separable cell division and vascular organisation outputs (Etchells *et al.*, 2013).

If TDIF does not confer positional information through PXY, the cavity hypothesis also does not explain the loss of organisation in ectopic *CLE41* overexpression lines or the observation that both periclinal and anticlinal divisions occur in plants subjected to TDIF treatment in liquid culture (Hirakawa *et al.*, 2010), and the absence of loss of organisation when *CLE41* is overexpressed specifically from the phloem (Etchells & Turner, 2010b; Etchells *et al.*, 2015). However, one possibility is that loss of organisation in lines such as *35S::CLE41* may be a consequence of TDIF-PXY signalling also negatively regulating xylem differentiation (see above). Vascular cell divisions are thought to be aligned in part by mechanical signals. In classical experiments, organised cell divisions reminiscent of those in the cambium were observed when tobacco pith (soft tissue at the centre of the stem) was subjected to mechanical stimulation (Figure 5)(Lintilhac & Vesecky, 1984). Xylem cells have large secondary cell walls that provide the plant with the majority of its mechanical strength, but also provide a rigid tissue against which the dividing cells and their daughters are positioned.

One possibility is that the altered xylem differentiation programme in lines such as those present in *35S::CLE41* alters mechanical signals conferred to the dividing cells, which could alter the orientation of cell division. Such changes in xylem differentiation may not be observed in *SUC2::CLE41* as TDIF would remain derived from the phloem and therefore reduced in cells on the xylem side of the procambium. Consequently, while the *pxy er* phenotype strongly supports the idea that vascular organisation is the consequence of an interaction between these two pathways, further work is required to assess the balance between differentiation in the xylem and phloem-derived positional information.

Interactions between PXY signalling and phytohormones

Initiation and elaboration of vascular tissue is complex and involves interactions between PXY and other signalling pathways. These interactions include those described above between PXY and ER that specifies vascular organisation (Etchells *et al.*, 2013), and control of xylem identity through GSK3's (Kondo *et al.*, 2014) which are also influenced by BR. However, a number of other studies have suggested that PXY also interacts to some degree with auxin, ethylene and jasmonate signalling.

Auxin signalling in particular has been widely associated with controlling vascular initiation in the embryo and root through the transcription factor *MONOPTEROS* (*MP*) (Berleth & Jurgens, 1993; Hardtke & Berleth, 1998) which, in turn, regulates expression of the helix-loop-helix (bHLH) transcription factor *TARGET OF MONOPTEROS5* (*TMO5)*. The TMO5 protein dimerizes with another bHLH transcription factor called LONESOME HIGHWAY (LHW) and this dimer then controls the number of periclinal cell divisions in the early vasculature (Ohashi-Ito & Bergmann, 2007; Schlereth *et al.*, 2010; De Rybel *et al.*, 2013). In addition to its roles in early patterning of vascular tissues, auxin has also been associated with initiation of secondary growth. Secondary growth is defined as radial expansion of plant stems and is typically the consequence of cell divisions in the vascular cambium. In *Arabidopsis*, secondary growth occurs in both the hypocotyl (Chaffey *et al.*, 2002) where vascular tissue is formed in a continuous ring (Figure 4A), in roots (Dolan & Roberts, 1995), and at the base of inflorescence stems where interfasicular cambium forms between vascular bundles (Sehr *et al.*, 2010). Auxin is transported in a basipetal manner in stems, so localised treatment of *Arabidopsis* stems with NPA, an auxin transport inhibitor, results in auxin accumulation above the site of NPA application. This build-up of auxin can induce an early onset of secondary growth. Strikingly, *WOX4* expression was shown to be induced at the site of auxin accumulation, and was subsequently demonstrated to be required for secondary

growth in *Arabidopsis* (Suer *et al.*, 2011). *WOX4* expression can be induced above sites of NPA application even in a *pxy* mutant background demonstrating that auxin regulates *WOX4* in a *PXY*-independent fashion. These results therefore suggest that *WOX4* may be one point of integration between PXY and auxin signalling.

The interaction between PXY and auxin signalling is not limited to regulation of *WOX4* expression in the vascular tissue. Auxin is critical in defining the sites of lateral root initiation in the root (Casimiro *et al.*, 2001), and surprisingly a recent study connects auxin, BIN2 and PXY in regulating this process (Cho *et al.*, 2014). Auxin response transcription factors *ARF7* and *ARF19* have previously been shown to promote lateral root initiation (Wilmoth *et al.*, 2005; Okushima *et al.*, 2007). AUX/IAA proteins act as repressors of ARF transcription factors in the absence of auxin. BIN2-mediated phosphorylation reduces the affinity of the interaction between ARF7/19 and AUX/IAAs. TDIF/PXY influence on the BIN2 GSK3 has been described above in the context of xylem differentiation, and evidence suggests that ARF7/19 phosphorylation via an interaction with BIN2 is PXY-regulated (Cho *et al.*, 2014). In support of this hypothesis, *pxy* mutants were shown to demonstrate reductions in lateral root density (Cho *et al.*, 2014), and in numbers of total emerged lateral roots (Figure 6). Reporter lines suggest that *PXY* is expressed in the root (Fisher & Turner, 2007) including at sites of lateral root initiation,(Cho *et al.*, 2014). Interestingly, one of the earliest steps of lateral root initiation is a periclinal division that occurs in the pericycle (Dubrovsky *et al.*, 2000), i.e. a division perpendicular to the radial axis of the stem that, superficially at least, is similar in its orientation to cell divisions in the endodermis in above ground parts of the plant that are required to set up the interfasicular cambium.

In addition to auxin, jasmonate (JA) signalling has also been shown to regulate secondary growth of plant stems. While jasmonate signalling is typically associated with plant defence responses (Ballaré, 2011), factors involved in jasmonate signalling demonstrate higher expression in stems that have a developed cambium than stems where the cambium has not initiated. Plants treated with JA demonstrate increases in secondary growth, and conversely, mutants unable to respond to JA demonstrate secondary growth reductions. (Sehr *et al.*, 2010). Transcription factors that respond to JA stimulus include members of the *ERF* transcription factor family (Nemhauser *et al.*, 2006; Pauwels *et al.*, 2008). A second factor implicated with growth in the cambium via control of expression of members of the *ERF* family is the gaseous hormone ethylene (Büttner & Singh, 1997; Fujimoto *et al.*, 2000; Lorenzo *et al.*, 2003; Nemhauser *et al.*, 2006). Ethylene has for many years been associated with thigmomorphogenesis – the response to mechanical perturbation (Leopold et al., 1972).

The formation of tension wood, which is formed in trees in response to gravitational stimulus is just such a response, and has also been shown to involve cell division in the cambium. Tree stems subjected to exposure of ethylene gas demonstrate considerable increases in radial growth consistent with ethylene signalling promoting vascular cell division (Leopold *et al.*, 1972; Brown & Leopold, 1973; Savidge, 1988; Love *et al.*, 2009; Vahala *et al.*, 2013). Furthermore, *Arabidopsis* mutants that demonstrate increases in ethylene production (*ethylene overproducer*; *eto1* and *eto2* mutants)(Chae *et al.*, 2003), also demonstrate increases in radial growth of the hypocotyl (Figure 7), presumably via an increase in cell division in the cambium (Etchells *et al.*, 2012).

ERF transcription factors, regulated by JA and ethylene are connected to PXY signalling because one of the hallmarks of the *pxy* mutant transcriptome is increases in *ERF* expression (Etchells *et al.*, 2012). For example *ERF1* and *ERF11* genes, shown to be ethylene responsive (Solano *et al.*, 1998; Fujimoto *et al.*, 2000; Alonso *et al.*, 2003; Nemhauser *et al.*, 2006), demonstrate increases in expression in *pxy* mutant stems. *ERF018* (also referred to as *ORA47*) and *ERF109* (also known as *RRTF*) are also upregulated in *pxy* and have been reported to be jasmonate-responsive (Pauwels *et al.*, 2008; Cai *et al.*, 2014). Evidence suggests that these *ERF*s promote vascular cell division as *erf* mutants demonstrate reductions in radial growth and vascular bundle size. Consequently, as *ERF*s promote vascular cell division and are upregulated in *pxy* mutants, these transcription factors are thought to represent a mechanism that compensates for the loss of cell division in *pxy* lines. This hypothesis is supported by *pxy erf109 erf018* and *pxy erf109 erf1* triple mutants, which demonstrate enhancements of cell division defects over double and single mutants. Increases in radial growth observed in *eto1* mutants are dependent on several of the *ERF* transcription factors also upregulated in *pxy* lines. This suggests that crosstalk exists between PXY and ethylene signalling and is supported by observations that *pxy* mutants are enhanced by mutants in the ethylene signal transduction pathway (Etchells *et al.*, 2012). While interactions between *PXY* and JA signalling have not been tested genetically, it is striking that genes upregulated in *pxy* mutants are also thought to be jasmonate-responsive. A complex picture of crosstalk between JA, ethylene and PXY signalling in the regulation of vascular cell division requires further testing.

Known unknowns

While the manner in which PXY signalling influences vascular development has been the subject of much research since the discovery of TDIF almost a decade ago, many questions

remain. Neither currently known transcription factor targets, nor signalling components completely explain the phenotypes observed in the loss of function receptor mutants. Consequently further factors involved in signal transduction, and targets of TDIF-PXY are yet to be identified. For example, one phenotype identified in the original *pxy* mutant was a change in the ratio of cell types present in the xylem. *pxy* mutants demonstrated an increase in the number fibre cells relative to vessels when compared to the wild type (Fisher & Turner, 2007). This suggests that PXY may also have an influence on the identity of cells leaving the cambium that form xylem cells, beyond its role of negative regulation of xylem identity in the procambium. It is rather striking that in mRNA profiles taken across the wood forming tissue of hybrid aspen that *PtPXY* (also referred to as *PtRLK1*) expression persists in the xylem (Schrader *et al.*, 2004).

Further questions also remain regarding genes that specify the TDIF ligand. Of the three genes that specify TDIF, only a lesion in *CLE41* has been identified. *cle41* mutants have fewer vascular cells but no defects in vascular organisation (Hirakawa *et al.*, 2008). In *cle41* lines, *CLE44* and *CLE42* may act to maintain vascular organisation, although another possibility is that interactions exist between PXY and other signals may control this output. The interaction between PXY and ER signalling has been described above, but interestingly, growth of plants in the presence of the CLE6 peptide has been shown to enhance the phenotype of TDIF-grown seedlings. This putative interaction may point to further levels of regulation at the cell membrane (Whitford *et al.*, 2008).

While the identification of factors that regulate various aspects of PXY signalling outputs has been successful, it remains unclear as to how these complex outputs are integrated. Consequently, the extent of transcriptional outputs of TDIF-PXY signalling, its interactions with other signalling modules and how these outputs interact in a complex network to specify vascular development are matters for ongoing research.

Plant biomass is a huge renewable resource of biofuels and biomaterials, and the major constituent of plant biomass is xylem derived from the vascular meristem. Targets were set in both Europe and the US for the uptake of biofuels particularly in transport fuel, and one obstacle to meeting these targets has been a requirement for a huge amount of plant biomass from a number of sources (Somerville, 2006). Manipulating plant vascular development is one possible way to increase available plant biomass, and modulation of TDIF-PXY signalling in Arabidopsis and aspen has been shown to considerably increase height, dry weight and stem diameter (Etchells *et al.*, 2015). A major challenge that remains is determination of how vascular development networks, including those influenced by TDIF-

PXY signalling, control biomass deposition in monocots, particularly for biomass/bioenergy crops such as miscanthus (Heaton *et al.*, 2008; Robson *et al.*, 2013), switchgrass (McLaughlin & Adams Kszos, 2005; Schmer *et al.*, 2008) and sorghum (Rooney *et al.*, 2007; Mullet *et al.*, 2014).

Materials and Methods

Staining of vascular bundles in Figure 2 was performed on transverse hand sections from the base of 10 cm wild type and *pxy* inflorescence stems. Plants were 4 weeks old and grown under long day conditions. Fresh sections were incubated for 5 min in 2% phloroglucinol; 95% ethanol prior to transfer to 3M HCl for 5 mins and mounted in 80% glycerol.

For analysis of lateral root initiation in wild type and *pxy* mutants (Figure 6), seeds were sorted for size (250-300um) and sterilized using a dichloroisocyanuric acid wash method prior to sowing on MS agar plates, where they were stratified at 4 °C for 2 days. Plates were placed vertically in 16 hour day growth chamber. 9 days after germination plates were scanned and lateral roots were counted for both genotypes. A T-test was performed to determine significance.

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Figure Legends

Figure 1. Morphology of *pxy* and *35S::CLE41* vascular bundles

Vascular bundles in transverse section from the base of the inflorescence stem of 8 week old plants of wild type (A), *35S::CLE41* (B), and *pxy* (C). Left hand panels show overall vascular bundle morphology; panels on the right show vascular tissue in close up. Phloem, procambium and xylem are marked with ph, pc and x respectively. Asterisks in (A) mark an organised cell file. Arrowheads in (B) and (C) mark cells intercalated with xylem that are not present in wild type. Scales are 50 μM (left panels) and 20 μM (right panels).

Figure 2. Lignin deposition in *pxy* mutants

Phloroglucinol stains lignols that are mostly present in lignin polymers in the secondary cell walls. Sections shown are from the base of 4 week old wild type (A), and *pxy* (B) inflorescence stems. In upper panels x marks xylem; ph marks the phloem; if marks interfascicular fibres. Arrowheads mark areas of the xylem where lignified secondary cell walls appear absent in wild type. These areas are reduced in *pxy*. Lower panels show close-ups with 'v' marking vessels. Arrows point to xylem parenchyma (p) which appear lignified in *pxy* mutants but not in wild type. Scales are 50 μM (upper panels) and 25 μM (lower).

Figure 3. Model showing BIN2 interactions that control vascular development

In the procambium (left panel) TDIF binds to PXY resulting in an interaction between PXY and BIN2, which in turn results in phosphorylation and proteolysis of the TF BES1. In contrast in the xylem (right panel), brassinosteroid binding to BRI receptors negatively regulates BIN2, preventing its phosphorylation. BES1 is therefore free to move to the nucleus and promote xylem differentiation.

Figure 4. Morphology of *pxy* and *er* mutant hypocotyls

Transverse sections of Col (A), *pxy* (B), *er* (C) and *pxy er* (D) hypocotyls. Upper panels show toluidine blue stained sections; lower panels aniline blue sections. In wild type (A) and *er* mutants (C), xylem are present in an organised vascular ring. Organisation is lost in *pxy* (B) and this phenotype is enhanced in *er pxy* (D). Organisation defects are characterised by intercalation of xylem and phloem, clearly seen in lower panels. Arrowheads mark phloem sieve plates (stained green with aniline blue) in positions where only xylem forms in wild type. Scales are 50 μM. Reprinted from Development 140:2224-2234.

Figure 5. Mechanical signals may align vascular cell divisions

(A) Tobacco pith explant prior to transfer into sterile culture. After transfer, the pith explant has an apparently random pattern of cell division (B), except in regions where pressure is applied (C). Here cells form repeated coplanar divisions reminiscent of the vascular cambium. Reprinted by permission from Macmillan Publishers Ltd: Nature 307:363-364, copyright (1984).

Figure 6. Lateral root formation in *pxy* mutants

(A) 9 day old wild type and *pxy* seedlings grown on vertical plates showing differences in lateral root emergence. (B) Quantification of lateral root number.

Figure 7. Vascular tissue in *eto2* mutants

Vascular tissue from inflorescence stems of 10 week old wild type (A) and *eto2* mutants (B) in transverse section. *eto2* plans have more cells in vascular bundles (middle panels) and early onset of secondary growth in interfascicular (if) regions (upper and lower panels). Scales are 100, 50 and 25 μM in upper, middle and lower panels, respectively. Reprinted from PLoS Genet 8(11): e1002997.

References

Alonso JM, Stepanova AN, Leisse TJ, Kim CJ, Chen H, Shinn P, Stevenson DK, Zimmerman J, Barajas P, Cheuk R, Gadrinab C, Heller C, Jeske A, Koesema E, Meyers CC, Parker H, Prednis L, Ansari Y, Choy N, Deen H, Geralt M, Hazari N, Hom E, Karnes M, Mulholland C, Ndubaku R, Schmidt I, Guzman P, Aguilar-Henonin L, Schmid M, Weigel D, Carter DE, Marchand T, Risseeuw E, Brogden D, Zeko A, Crosby WL, Berry CC, Ecker JR. 2003. Genome-Wide Insertional Mutagenesis of Arabidopsis thaliana. *Science* **301**(5633): 653-657.

Ballaré CL. 2011. Jasmonate-induced defenses: a tale of intelligence, collaborators and rascals. *Trends in plant science* **16**(5): 249-257.

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**(3): 823-831.

Berleth T, Jurgens G. 1993. The role of the monopteros gene in organising the basal body region of the Arabidopsis embryo. *Development* **118**(2): 575-587.

Brand U, Fletcher JC, Hobe M, Meyerowitz EM, Simon R. 2000. Dependence of stem cell fate in Arabidopsis an a feedback loop regulated by CLV3 activity. *Science* **289**(5479): 617-619.

Brown KM, Leopold AC. 1973. Ethylene and the Regulation of Growth in Pine. *Canadian Journal of Forest Research* **3**(1): 142-145.

Büttner M, Singh KB. 1997. Arabidopsis thaliana ethylene-responsive element binding protein (AtEBP), an ethylene-inducible, GCC box DNA-binding protein interacts with an ocs element bindingâ€‰protein. *Proceedings of the National Academy of Sciences of the United States of America* **94**(11): 5961-5966.

Cai XT, Xu P, Zhao PX, Liu R, Yu LH, Xiang CB. 2014. Arabidopsis ERF109 mediates cross-talk between jasmonic acid and auxin biosynthesis during lateral root formation. *Nat Commun* **5**: 5833.

Cano-Delgado A, Yin YH, Yu C, Vafeados D, Mora-Garcia S, Cheng JC, Nam KH, Li JM, Chory J. 2004. BRL1 and BRL3 are novel brassinosteroid receptors that function in vascular differentiation in Arabidopsis. *Development* **131**(21): 5341-5351.

Casimiro I, Marchant A, Bhalerao RP, Beeckman T, Dhooge S, Swarup R, Graham N, Inzé D, Sandberg G, Casero PJ, Bennett M. 2001. Auxin Transport Promotes Arabidopsis Lateral Root Initiation. *The Plant Cell* **13**(4): 843-852.

Chae HS, Faure F, Kieber JJ. 2003. The eto1, eto2, and eto3 Mutations and Cytokinin Treatment Increase Ethylene Biosynthesis in Arabidopsis by Increasing the Stability of ACS Protein. *The Plant Cell* **15**(2): 545-559.

Chaffey N, Cholewa E, Regan S, Sundberg B. 2002. Secondary xylem development in Arabidopsis: a model for wood formation. *Physiologia Plantarum* **114**(4): 594-600.

Cho H, Ryu H, Rho S, Hill K, Smith S, Audenaert D, Park J, Han S, Beeckman T, Bennett MJ, Hwang D, De Smet I, Hwang I. 2014. A secreted peptide acts on BIN2-mediated phosphorylation of ARFs to potentiate auxin response during lateral root development. *Nat Cell Biol* **16**(1): 66-76.

De Rybel B, Möller B, Yoshida S, Grabowicz I, Barbier de Reuille P, Boeren S, Smith Richard S, Borst Jan W, Weijers D. 2013. A bHLH Complex Controls Embryonic Vascular Tissue Establishment and Indeterminate Growth in Arabidopsis. *Developmental cell* **24**(4): 426-437.

Demura T, Tashiro G, Horiguchi G, Kishimoto N, Kubo M, Matsuoka N, Minami A, Nagata-Hiwatashi M, Nakamura K, Okamura Y, Sassa N, Suzuki S, Yazaki J, Kikuchi S, Fukuda H. 2002. Visualization by comprehensive microarray analysis of gene expression programs during transdifferentiation of mesophyll cells into xylem cells. *Proceedings of the National Academy of Sciences* **99**(24): 15794-15799.

DeYoung BJ, Bickle KL, Schrage KJ, Muskett P, Patel K, Clark SE. 2006. The CLAVATA1-related BAM1, BAM2 and BAM3 receptor kinase-like proteins are required for meristem function in Arabidopsis. *Plant Journal* **45**: 1-16.

Dolan L, Roberts K. 1995. Secondary thickening in roots of Arabidopsis thaliana: anatomy and cell surface changes. *New Phytologist* **131**(1): 121-128.

Dubrovsky JG, Doerner PW, Colón-Carmona A, Rost TL. 2000. Pericycle Cell Proliferation and Lateral Root Initiation in Arabidopsis. *Plant Physiology* **124**(4): 1648-1657.

Etchells JP, Mishra Laxmi S, Kumar M, Campbell L, Turner Simon R. 2015. Wood Formation in Trees Is Increased by Manipulating PXY-Regulated Cell Division. *Current Biology* **25**(8): 1050-1055.

Etchells JP, Provost CM, Mishra L, Turner SR. 2013. *WOX4* and *WOX14* act downstream of the PXY receptor kinase to regulate plant vascular proliferation independently of any role in vascular organisation. *Development* **140**: 2224-2234.

Etchells JP, Provost CM, Turner SR. 2012. Plant Vascular Cell Division Is Maintained by an Interaction between PXY and Ethylene Signalling. *PLoS Genetics* **8**(11): e1002997.

Etchells JP, Turner SR. 2010a. Orientation of vascular cell divisions in Arabidopsis. *Plant Signaling and Behaviour* **5**: 730-732.

Etchells JP, Turner SR. 2010b. The PXY-CLE41 receptor ligand pair defines a multifunctional pathway that controls the rate and orientation of vascular cell division. *Development* **137**(5): 767-774.

Fisher K, Turner S. 2007. PXY, a receptor-like kinase essential for maintaining polarity during plant vascular-tissue development. *Current Biology* **17**(12): 1061-1066.

Fletcher LC, Brand U, Running MP, Simon R, Meyerowitz EM. 1999. Signaling of cell fate decisions by CLAVATA3 in Arabidopsis shoot meristems. *Science* **283**(5409): 1911-1914.

Fujimoto SY, Ohta M, Usui A, Shinshi H, Ohme-Takagi M. 2000. Arabidopsis Ethylene-Responsive Element Binding Factors Act as Transcriptional Activators or Repressors of GCC Box-Mediated Gene Expression. *The Plant Cell* **12**(3): 393-404.

Fukuda H. 1997. Tracheary Element Differentiation. *The Plant Cell* **9**(7): 1147-1156.

Fukuda H, Komamine A. 1980. Establishment of an Experimental System for the Study of Tracheary Element Differentiation from Single Cells Isolated from the Mesophyll of Zinnia elegans. *Plant Physiology* **65**(1): 57-60.

Furuta KM, Hellmann E, Helariutta Y. 2014. Molecular Control of Cell Specification and Cell Differentiation During Procambial Development. *Annual Review of Plant Biology, Vol 65* **65**: 607-638.

Goldstein B. 1995. Cell contacts orient some cell division axes in the Caenorhabditis elegans embryo. *The Journal of Cell Biology* **129**(4): 1071-1080.

Hanzawa Y, Takahashi T, Michael AJ, Burtin D, Long D, Pineiro M, Coupland G, Komeda Y. 2000. ACAULIS5, an Arabidopsis gene required for stem elongation, encodes a spermine synthase. *Embo Journal* **19**(16): 4248-4256.

Hardtke CS, Berleth T. 1998. The Arabidopsis gene MONOPTEROS encodes a transcription factor mediating embryo axis formation and vascular development. *Embo Journal* **17**(5): 1405-1411.

Heaton EA, Dohleman FG, Long SP. 2008. Meeting US biofuel goals with less land: the potential of Miscanthus. *Global Change Biology* **14**(9): 2000-2014.

Hirakawa Y, Kondo Y, Fukuda H. 2010. TDIF Peptide Signaling Regulates Vascular Stem Cell Proliferation via the WOX4 Homeobox Gene in Arabidopsis. *Plant Cell* **22**(8): 2618-2629.

Hirakawa Y, Shinohara H, Kondo Y, Inoue A, Nakanomyo I, Ogawa M, Sawa S, Ohashi-Ito K, Matsubayashi Y, Fukuda H. 2008. Non-cell-autonomous control of vascular stem cell fate by a CLE peptide/receptor system. *Proceedings of the National Academy of Sciences, USA* **105**: 15208-15213.

Ito Y, Nakanomyo I, Motose H, Iwamoto K, Sawa S, Dohmae N, Fukuda H. 2006. Dodeca-CLE peptides as suppressors of plant stem cell differentiation. *Science* **313**(5788): 842-845.

Jouannet V, Brackmann K, Greb T. 2015. (Pro)cambium formation and proliferation: two sides of the same coin? *Current Opinion in Plant Biology* **23**: 54-60.

Kondo T, Sawa S, Kinoshita A, Mizuno S, Kakimoto T, Fukuda H, Sakagami Y. 2006. A plant peptide encoded by CLV3 identified by in situ MALDI-TOF MS analysis. *Science* **313**(5788): 845-848.

Kondo Y, Ito T, Nakagami H, Hirakawa Y, Saito M, Tamaki T, Shirasu K, Fukuda H. 2014. Plant GSK3 proteins regulate xylem cell differentiation downstream of TDIF–TDR signalling. *Nat Commun* **5**.

Kubo M, Udagawa M, Nishikubo N, Horiguchi G, Yamaguchi M, Ito J, Mimura T, Fukuda H, Demura T. 2005. Transcription switches for protoxylem and metaxylem vessel formation. *Genes & Development* **19**(16): 1855-1860.

Laux T, Mayer KF, Berger J, Jurgens G. 1996. The WUSCHEL gene is required for shoot and floral meristem integrity in Arabidopsis. *Development* **122**(1): 87-96.

Leopold AC, Brown KM, Emerson FH. 1972. Ethylene in the Wood of Stressed Trees. *Horticultural Science* **7**: 175.

Li J, Nam KH. 2002. Regulation of Brassinosteroid Signaling by a GSK3/SHAGGY-Like Kinase. *Science* **295**(5558): 1299-1301.

Lintilhac PM, Vesecky TB. 1984. Stress-induced alignment of division plane in plant tissues grown in vitro. *Nature* **307**(5949): 363-364.

Lorenzo O, Piqueras R, Sanchez-Serrano JJ, Solano R. 2003. ETHYLENE RESPONSE FACTOR1 Integrates Signals from Ethylene and Jasmonate Pathways in Plant Defense. *The Plant Cell* **15**(1): 165-178.

Love J, Björklund S, Vahala J, Hertzberg M, Kangasjärvi J, Sundberg B. 2009. Ethylene is an endogenous stimulator of cell division in the cambial meristem of Populus. *Proceedings of the National Academy of Sciences, USA* **106**(14): 5984-5989.

Lucas WJ, Groover A, Lichtenberger R, Furuta K, Yadav S-R, Helariutta Y, He X-Q, Fukuda H, Kang J, Brady SM, Patrick JW, Sperry J, Yoshida A, Lopez-Millan A-F, Grusak MA, Kachroo P. 2013. The Plant Vascular System: Evolution, Development and Functions. *Journal of Integrative Plant Biology* **55**(4): 294-388.

McConnell JR, Barton MK. 1995. Effect of mutations in the *PINHEAD* gene of *Arabidopsis* on the formation of shoot apical meristems. *Developmental Genetics* **16**(4): 358-366.

McLaughlin SB, Adams Kszos L. 2005. Development of switchgrass (Panicum virgatum) as a bioenergy feedstock in the United States. *Biomass and Bioenergy* **28**(6): 515-535.

Mullet J, Morishige D, McCormick R, Truong S, Hilley J, McKinley B, Anderson R, Olson S, Rooney W. 2014. Energy Sorghum—a genetic model for the design of C4 grass bioenergy crops. *Journal of Experimental Botany*.

Nemhauser JL, Hong F, Chory J. 2006. Different Plant Hormones Regulate Similar Processes through Largely Nonoverlapping Transcriptional Responses. *Cell* **126**: 467-475.

Oelkers K, Goffard N, Weiller G, Gresshoff P, Mathesius U, Frickey T. 2008. Bioinformatic analysis of the CLE signaling peptide family. *BMC Plant Biology* **8**(1): 1.

Ogawa M, Shinohara H, Sakagami Y, Matsubayashi Y. 2008. Arabidopsis CLV3 peptide directly binds CLV1 ectodomain. *Science* **319**(5861): 294-294.

Ohashi-Ito K, Bergmann DC. 2007. Regulation of the Arabidopsis root vascular initial population by LONESOME HIGHWAY. *Development* **134**(16): 2959-2968.

Ohashi-Ito K, Fukuda H. 2014. Initiation of vascular development. *Physiologia Plantarum* **151**(2): 142-146.

Okushima Y, Fukaki H, Onoda M, Theologis A, Tasaka M. 2007. ARF7 and ARF19 Regulate Lateral Root Formation via Direct Activation of LBD/ASL Genes in Arabidopsis. *The Plant Cell* **19**(1): 118-130.

Pauwels L, Morreel K, De Witte E, Lammertyn F, Van Montagu M, Boerjan W, Inzé D, Goossens A. 2008. Mapping methyl jasmonate-mediated transcriptional reprogramming of metabolism and cell cycle progression in cultured Arabidopsis cells. *Proceedings of the National Academy of Sciences USA* **105**(4): 1380-1385.

Pineau P, Freydier A, Ranocha P, Jauneau A, Turner S, Lemonnier G, Renou J-P, Tarkowski P, Sandberg G, Jouanin L, Sundberg B, Boudet A-M, Goffner G, Pichon M. 2005. *hca*: an

Arabidopsis mutant exhibiting unusual cambial activity and altered vascular patterning. *Plant Journal* **44**: 271-289.

Robson P, Jensen E, Hawkins S, White SR, Kenobi K, Clifton-Brown J, Donnison I, Farrar K. 2013. Accelerating the domestication of a bioenergy crop: identifying and modelling morphological targets for sustainable yield increase in Miscanthus. *Journal of Experimental Botany* **64**(14): 4143-4155.

Rooney WL, Blumenthal J, Bean B, Mullet JE. 2007. Designing sorghum as a dedicated bioenergy feedstock. *Biofuels, Bioproducts and Biorefining* **1**(2): 147-157.

Savidge RA. 1988. Auxin and ethylene regulation of diameter growth in trees. *Tree Physiology* **4**: 401-414.

Schlereth A, Moller B, Liu W, Kientz M, Flipse J, Rademacher EH, Schmid M, Jurgens G, Weijers D. 2010. MONOPTEROS controls embryonic root initiation by regulating a mobile transcription factor. *Nature* **464**(7290): 913-916.

Schlesinger A, Shelton C, Maloof JN, Meneghini M, Bowerman B. 1999. Wnt pathway components orient a mitotic spindle in the early *Caenorhabitis elegans* embryo without requiring gene transcription in the responding cell. *Genes & Development* **13**: 2028-2038.

Schmer MR, Vogel KP, Mitchell RB, Perrin RK. 2008. Net energy of cellulosic ethanol from switchgrass. *Proceedings of the National Academy of Sciences* **105**(2): 464-469.

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**(6): 635-644.

Schrader J, Nilsson J, Mellerowicz E, Berglund A, Nilsson P, Hertzberg M, Sandberg G. 2004. A High-Resolution Transcript Profile across the Wood-Forming Meristem of Poplar Identifies Potential Regulators of Cambial Stem Cell Identity. *Plant Cell* **16**(9): 2278-2292.

Sehr EM, Agusti J, Lehner R, Farmer EE, Schwarz M, Greb T. 2010. Analysis of secondary growth in the Arabidopsis shoot reveals a positive role of jasmonate signalling in cambium formation. *The Plant Journal* **63**(5): 811-822.

Shiu S-H, Bleecker AB. 2001. Receptor-like kinases from Arabidopsis form a monophyletic gene family related to animal receptor kinases. *Proceedings of the National Academy of Sciences, USA* **98**(19): 10763-10768.

Shpak ED, Berthiaume CT, Hill EJ, Torii KU. 2004. Synergistic interaction of three ERECTA-family receptor-like kinases controls Arabidopsis organ growth and flower development by promoting cell proliferation. *Development* **131**(7): 1491-1501.

Shpak ED, McAbee JM, Pillitteri LJ, Torii KU. 2005. Stomatal Patterning and Differentiation by Synergistic Interactions of Receptor Kinases. *Science* **309**(5732): 290-293.

Solano R, Stepanova A, Chao Q, Ecker JR. 1998. Nuclear events in ethylene signaling: a transcriptional cascade mediated by ETHYLENE-INSENSITIVE3 and ETHYLENE-RESPONSE-FACTOR1. *Genes & Development* **12**(23): 3703-3714.

Somerville C. 2006. The Billion-Ton Biofuels Vision. *Science* **312**(5778): 1277.

Stahl Y, Wink RH, Ingram GC, Simon R. 2009. A Signaling Module Controlling the Stem Cell Niche in Arabidopsis Root Meristems. *Current Biology* **19**(11): 909-914.

Strabala TJ, O'Donnell PJ, Smit AM, Ampomah-Dwamena C, Martin EJ, Netzler N, Nieuwenhuizen NJ, Quinn BD, Foote HCC, Hudson KR. 2006. Gain-of-function phenotypes of many CLAVATA3/ESR genes, including four new family members, correlate with tandem variations in the conserved CLAVATA3/ESR domain. *Plant Physiology* **140**(4): 1331-1344.

Suer S, Agusti J, Sanchez P, Schwarz M, Greb T. 2011. WOX4 Imparts Auxin Responsiveness to Cambium Cells in Arabidopsis. *The Plant Cell* **23**(9): 3247-3259.

Taylor NG, Scheible WR, Cutler S, Somerville CR, Turner SR. 1999. The irregular xylem3 locus of arabidopsis encodes a cellulose synthase required for secondary cell wall synthesis. *Plant Cell* **11**(5): 769-779.

Uchida N, Tasaka M. 2013. Regulation of plant vascular stem cells by endodermis-derived EPFL-family peptide hormones and phloem-expressed ERECTA-family receptor kinases. *Journal of Experimental Botany*.

Vahala J, Felten J, Love J, Gorzsas A, Gerber L, Lamminmaki A, Kangasjarvi J, Sundberg B. 2013. A genome-wide screen for ethylene-induced ethylene response factors (ERFs) in hybrid aspen stem identifies ERF genes that modify stem growth and wood properties. *New Phytol* **200**(2): 511-522.

Whitford R, Fernandez A, De Groodt R, Ortega E, Hilson P. 2008. Plant CLE peptides from two distinct functional classes synergistically induce division of vascular cells. *Proceedings of the National Academy of Sciences USA* **105**(47): 18625-18630.

Wilmoth JC, Wang S, Tiwari SB, Joshi AD, Hagen G, Guilfoyle TJ, Alonso JM, Ecker JR, Reed JW. 2005. NPH4/ARF7 and ARF19 promote leaf expansion and auxin-induced lateral root formation. *The Plant Journal* **43**(1): 118-130.

Xu J, Zhang S. 2015. Mitogen-activated protein kinase cascades in signaling plant growth and development. *Trends in plant science* **20**(1): 56-64.

Yamamoto R, Fujioka S, Demura T, Takatsuto S, Yoshida S, Fukuda H. 2001. Brassinosteroid Levels Increase Drastically Prior to Morphogenesis of Tracheary Elements. *Plant Physiology* **125**(2): 556-563.

Yamamoto R, Fujioka S, Iwamoto K, Demura T, Takatsuto S, Yoshida S, Fukuda H. 2007. Co-regulation of brassinosteroid biosynthesis-related genes during xylem cell differentiation. *Plant & Cell Physiology* **48**(1): 74-83.

Yin Y, Vafeados D, Tao Y, Yoshida S, Asami T, Chory J. 2005. A New Class of Transcription Factors Mediates Brassinosteroid-Regulated Gene Expression in Arabidopsis. *Cell* **120**(2): 249-259.

Yin Y, Wang Z-Y, Mora-Garcia S, Li J, Yoshida S, Asami T, Chory J. 2002. BES1 Accumulates in the Nucleus in Response to Brassinosteroids to Regulate Gene Expression and Promote Stem Elongation. *Cell* **109**(2): 181-191.

Youn J-H, Kim T-W. 2015. Functional Insights of Plant GSK3-like Kinases: Multi-Taskers in Diverse Cellular Signal Transduction Pathways. *Molecular Plant* **8**(4): 552-565.

Figure 1

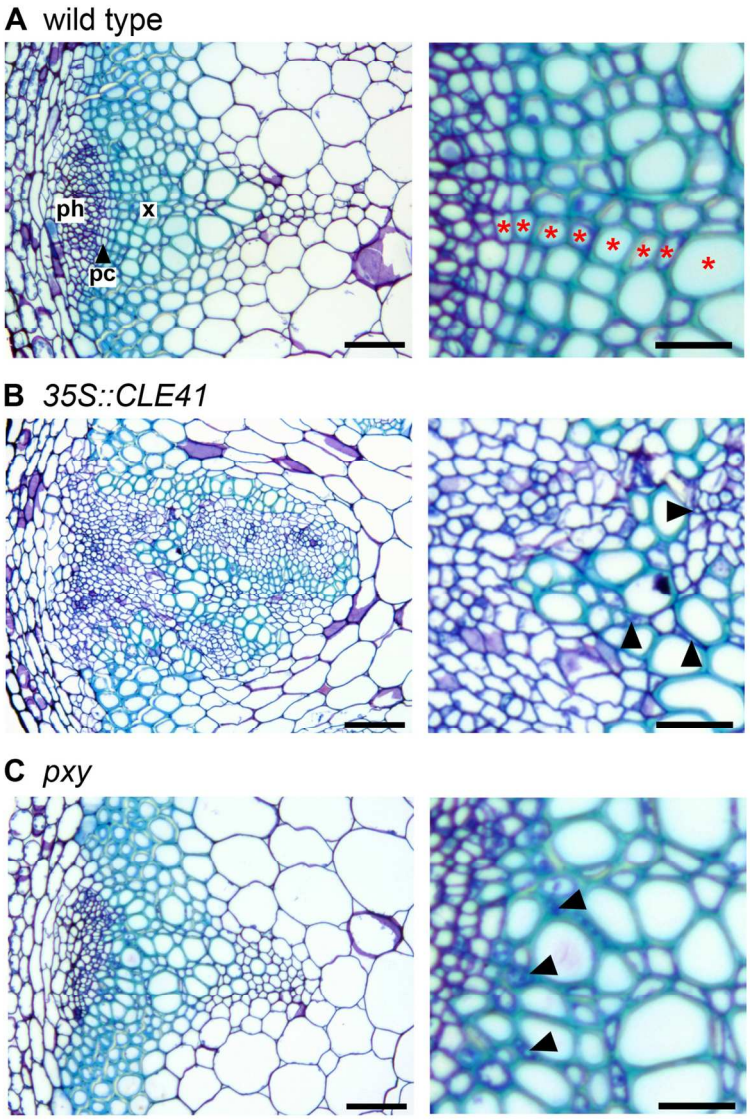

Figure 1
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Figure 2

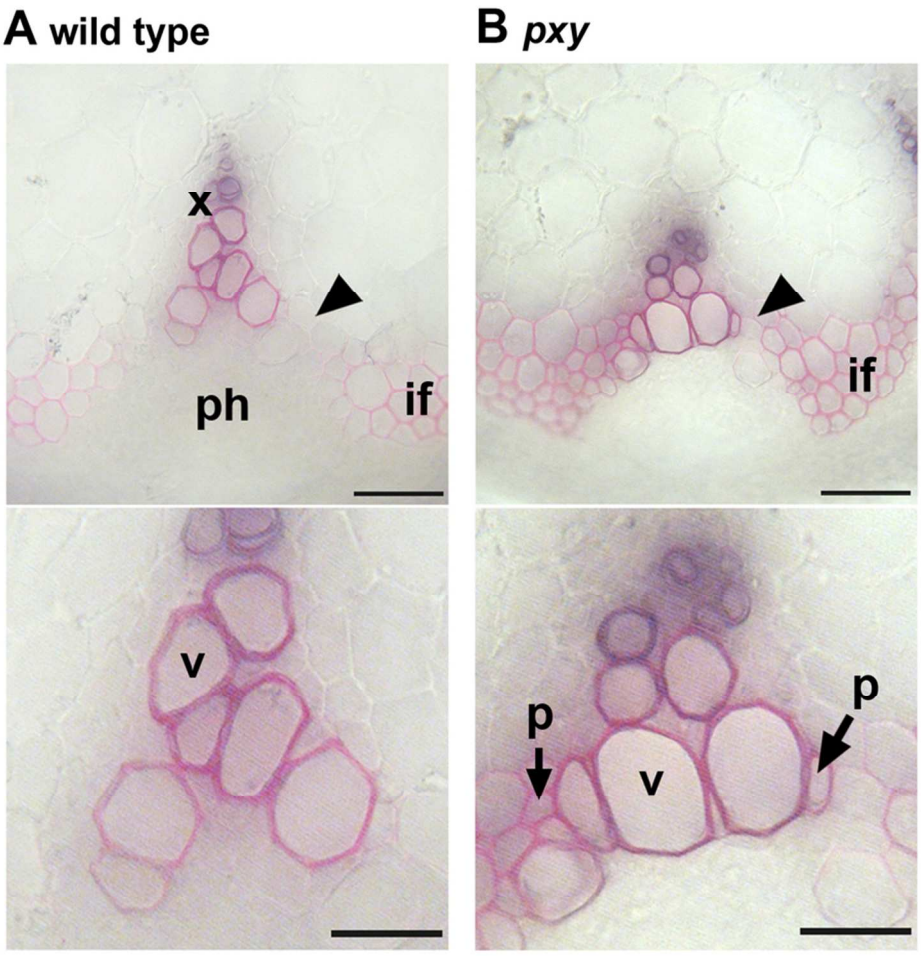

Figure 2
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Figure 3

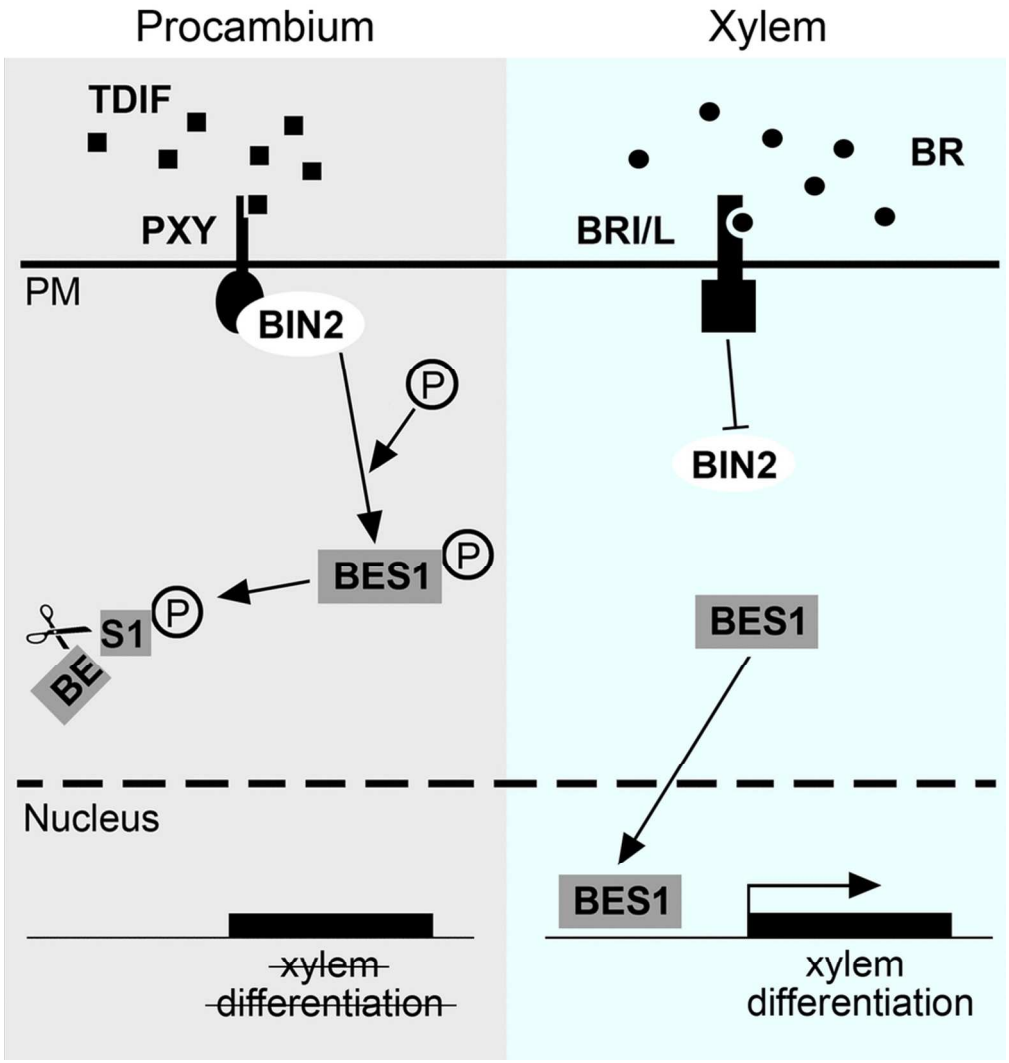

Figure 3
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Figure 4

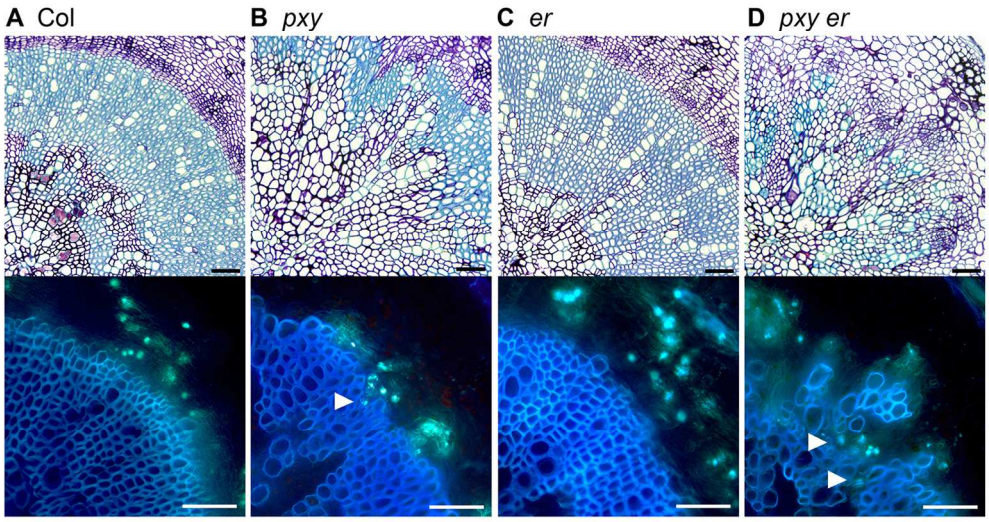

Figure 4
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Figure 5

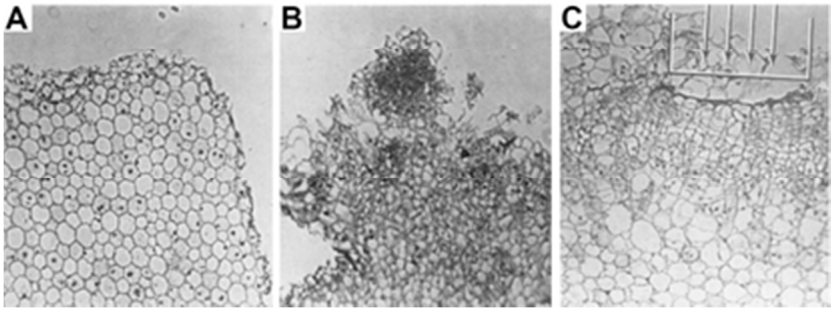

Figure 5
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Figure 6

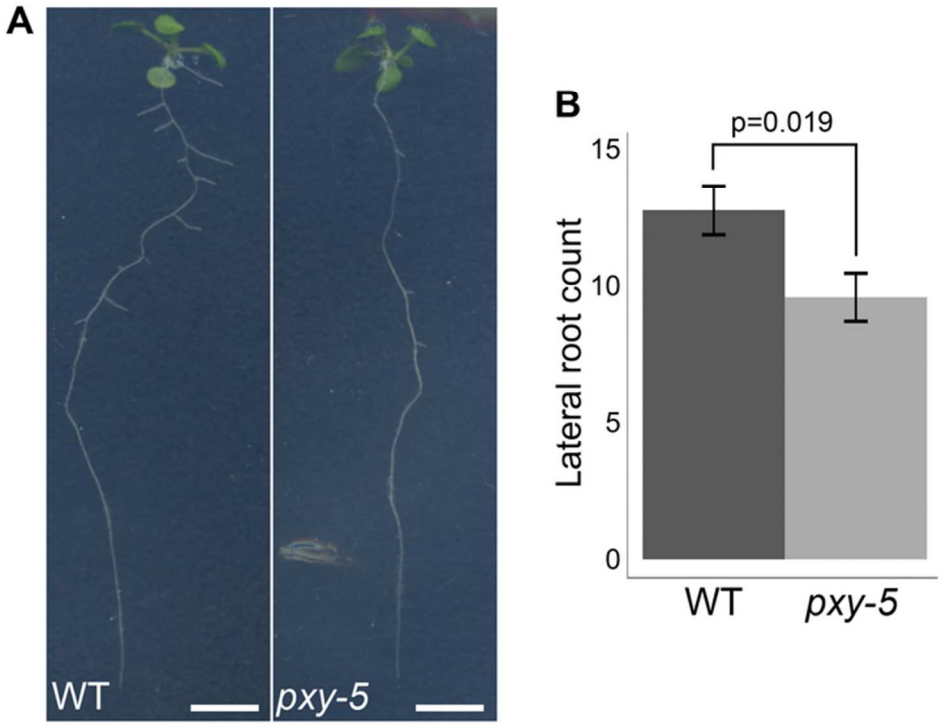

Figure 6
64x56mm (300 x 300 DPI)

Figure 7

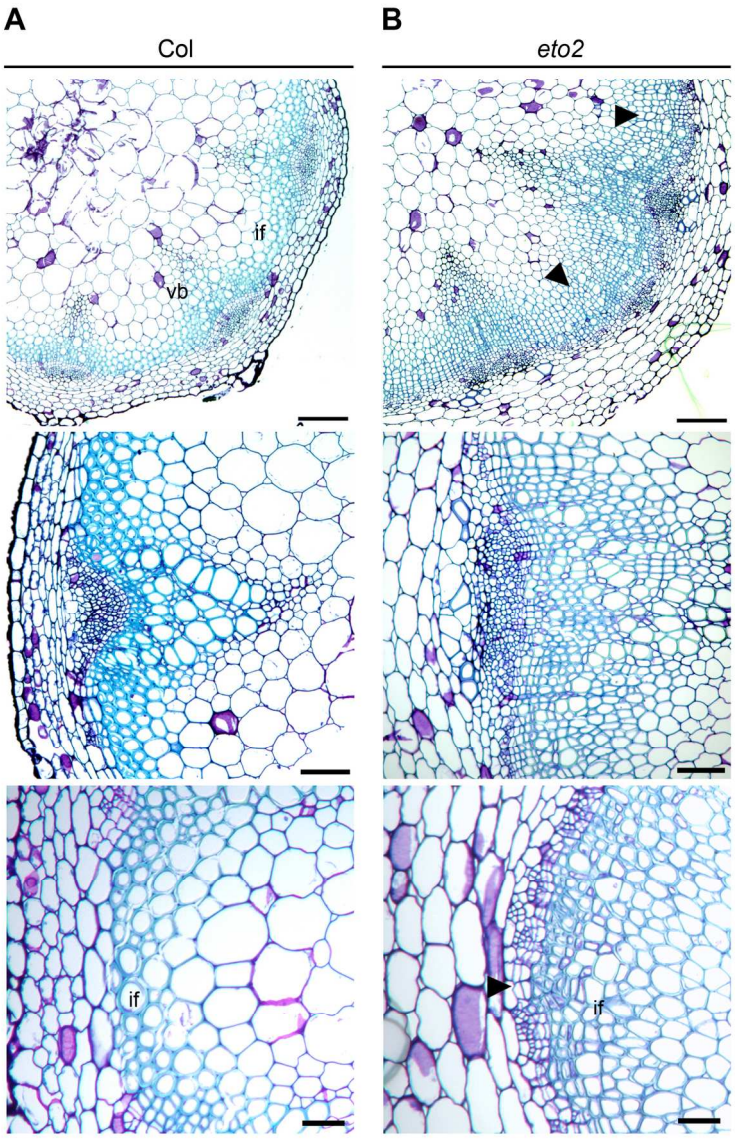

Figure 7
139x217mm (300 x 300 DPI)