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Repressor for Hire! The vital roles of TOPLESS-mediated transcriptional repression in plants

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Summary

Transcriptional corepressors play important roles in establishing the appropriate levels of gene expression during growth and development. The TOPLESS (TPL) family of corepressors are critical for all plant life. TPLs are involved in numerous developmental processes and in the response to extrinsic challenges. As such these proteins have been the focus of intense study since Long and colleagues first described the TPL corepressor in 2006. In this review we will explore the evolutionary history of these essential plant-specific proteins, their mechanism of action based on recent structural analyses, and the myriad of pathways in which they function. We speculate how relatively minor changes in the peptide sequence of transcriptional regulators allowed them to recruit TPL into new processes, driving innovation and resulting in TPL becoming vital for plant development.

Key words: corepressor, EAR motif, repression domain, TOPLESS, gene expression, adaptor proteins, hormone signalling, *Arabidopsis thaliana*

Introduction

Normal growth and development are the products of strictly regulated gene expression, requiring a balance between transcriptional activation and repression. Consequently, mechanisms that switch off gene expression are just as important as those that turn it on. As part of a multifaceted regulatory system, corepressor proteins mediate transcriptional repression of specific target genes. Corepressors function as part of large multiprotein complexes that govern access by the transcriptional machinery to specific loci by modifying chromatin structure. Many eukaryotic corepressors associate with histone deacetylase (HDAC) activity which generates condensed and inaccessible chromatin, typically associated with transcriptional repression (reviewed by Lee & Golz, 2012). Corepressors provide the link between DNA-recognition specificity, provided by transcription factors (TFs), and the enzymatic activity of chromatin remodellers. Corepressors cannot bind DNA independently, so are reliant on direct or indirect interactions with specific TFs. An important family of eukaryotic corepressors are the Groucho (Gro)/Tup1 proteins, which function in combination with class I HDACs to modify chromatin and inhibit target gene expression (Jennings and Ish-Horowicz, 2008).

In this review we will discuss how a plant family of Gro/Tup1 corepressors, TOPLESS (TPL)/TOPLESS-RELATED (TPR), has been co-opted into multiple pathways to become fundamental to almost all aspects of plant development. We will touch on their evolutionary history, their structure, and their mechanism of action, and we will highlight outstanding questions in this rapidly expanding field.

TOPLESS is a GRO/Tup1-like corepressor

One of a family of five in *Arabidopsis thaliana*, TOPLESS (TPL) is a conserved corepressor (Martin-Arevalillo et al., 2017; Long et al., 2006), which derives its name from the striking phenotype of the dominant-negative *tpl-1* mutant, in which a single amino acid substitution within a highly conserved region of the protein (N176H; red star in Fig. 1) causes dramatic temperature-sensitive abnormalities in growth and development (Long et al., 2002; Long et al., 2006). *tpl-1* plants show a range of morphological alterations, the most severe being conversion of the embryonic shoot into a second root. Smith and Long (2010) demonstrated that this phenotype was caused by expansion of *PLETHORA* (*PLT*) gene expression into the apical domain. *PLT* genes are master regulators of basal fate normally repressed by TPL in apical tissues (Smith & Long, 2010). However, the pleiotropic phenotype of *tpl-1* implied that TPL had a broader regulatory role. It has since been shown that TPL/TPR proteins participate in numerous other processes throughout plant development. TPL/TPRs resemble Gro/Tup1 corepressors found in other eukaryotes (Long et al., 2006), such as Gro from fruit flies, Tup1 from yeast and the human Transducin-Linked Enhancer of Split (TLE) and Transducin (Beta)-Like 1X-Linked (TBL1) proteins (Lee & Golz, 2012). In addition to TPL/TPR, plants have a second family of Gro/Tup1-related corepressors (LEUNIGs) with various roles in development, reviewed elsewhere (Lee & Golz, 2012). TPL/TPR corepressors have an analogous role to animal and yeast Gro/Tup1 corepressors, and in common with them they couple TFs to class I HDACs to repress the expression of target genes (Krogan et al., 2012). TPL/TPRs and other Gro/Tup1 proteins have similar domain structures, featuring amino-terminal protein-binding domains and carboxy-terminal clusters of WD40 motifs as common features (Lee & Golz, 2012) (Fig. 1). These WD40 motifs form β-propeller structures that are also employed in protein-protein interactions (Collins et al., 2019, Liu et al., 2019).

TOPLESS is recruited to target genes via Repression Domains

Repressive transcriptional regulators inhibit target gene expression either passively, by competing with activators on promoters, or actively. Active repressors possess short repression domain (RD) sequences that are responsible for recruiting corepressor proteins (Lee & Golz, 2012).The recruitment of TPL/TPRs to specific target genes occurs directly through interaction with RD-containing TFs or indirectly via specific RD-containing adaptor proteins that link the corepressor to TFs that lack an RD. Kieffer et al. (2006) demonstrated a functionally significant and direct interaction between TPL and the TF WUSCHEL (WUS), which was dependent on conserved RDs at the C-terminus of WUS, including an ERF-associated amphiphilic repression (EAR) motif (with sequence LELTL) known to be involved in transcriptional repression in plants (Ohta et al., 2001). An example of TPL/TPR recruitment by adaptor proteins is at the core of auxin signalling. AUX/IAA adaptor proteins associate with activating auxin-response transcription factors (ARFs) where they recruit TPL/TPRs to repress auxin responses (see below). The EAR-like motifs of AUX/IAAs are necessary to recruit TPL/TPRs (Szemenyei et al., 2008). EAR motifs, which follow the consensus sequence (L/F)DLN(L/F)xP or LxLxL, are one type of RD, and are found in at least 12% of transcription-related proteins in *Arabidopsis* (Kagale et al., 2010). Variants of the canonical EAR have been identified in other species, such as the TPL-recruiting LxLxPP sequence of AUX/IAA proteins from the bryophyte *Physcomitrium* (formerly *Physcomitrella*) *patens* Mitten (Causier et al., 2012a). Other unrelated RDs have also been shown to act in transcriptional repression and to recruit TPL, including the B3 repression domains (BRD; R/K/MLFGV or IKLFG) found in RAV, ARF, Hsf, MYB and CDF families (Ikeda & Ohme-Takagi, 2009; Causier et al., 2012b; Goralogia et al., 2017), the WUS-box (a second functionally significant RD in WUS, with sequence TLxLF; Ikeda et al., 2009), the PF(V/L)FL sequence in the miP1a/b microProtein adaptors (Graeff et al., 2016) and the A(L/I)W(L/V) sequence of the ROXY adaptors (Uhrig et al., 2017).

Recently, considerable progress has been made towards understanding the structural basis of EAR-type repression domain binding by TPL/TPRs and the transcriptional repression they mediate. TPL family proteins are composed of the amino-terminus TOPLESS Domain (TPD) and two clusters of carboxy-terminus WD40 repeats (Long et al., 2006; Ke et al., 2015) (Fig. 1A). The TPD contains three conserved domains: Lisencephaly Homologue (LisH), C-terminal to LisH (CTLH) and CT11-RanBPM (CRA) (Fig. 1B). The TPD is not only conserved at the sequence level, but also structurally (Ke et al., 2015; Martin-Arevalillo et al., 2017), and is necessary for interaction with RD sequences (Szemenyei et al., 2008). Structural data also revealed that RD peptides derived from a variety of different transcription-related proteins bound the same hydrophobic groove formed by the CTLH and CRA domains (Ke et al., 2015;

Martin-Arevalillo et al., 2017) (Fig. 1C). Crucially, Ke et al. (2015) showed that LxLxL EAR-family RDs have different affinities for the TPR2 TPD, suggesting that amino acids flanking the RD contribute to the interactions. Post-translational modification may also contribute to interactions. For example, SUMOylation of TPR1 negatively regulates its interaction with TFs involved in plant immune responses without reducing protein stability (Niu et al., 2019)

In 2019 it was revealed that transcription factors with BRD and DLNxxP type RDs (e.g. RAV-family TFs) bind to the C-terminal WD40 β-propellers of TPL proteins, rather than the TPD, and is RD-dependent (Collins et al., 2019; Liu et al., 2019). Interestingly, the substitution of a single, highly conserved amino acid in the first β-propeller of the maize TPL protein RAMOSA1 ENHANCER LOCUS 2 (REL2), inhibited interaction with these TFs (Liu et al., 2019). This key residue is predicted to lie on the top face of β-propeller 1, which is the region of Gro/TLE proteins that binds short peptide motifs found on diverse animal TFs, suggesting that this is a conserved mechanism of TF binding and an alternative path to recruiting TPL family proteins for transcriptional repression (Liu et al., 2019). Despite being close relatives, TPL and TPR1 show differential binding to RAV-family TFs, caused by sequence differences within their WD40 β-propellers, a discovery with implications for redundancy between TPL family members (Collins et al., 2019).

Interactions between TPL and proteins with RDs have been identified in taxa from algae through to angiosperms, suggesting that this repression mechanism has been evolutionarily conserved since before the earliest land plants (>450 million years) (Martin-Arevalillo et al., 2017). Changes to protein-protein interactions have been the catalyst for developmental innovation throughout evolution (Bartlett, 2020). TPL interactors are enriched for RDs (Causier et al., 2012b) and the acquisition of an RD, a comparatively small motif, may have enabled TFs to recruit the pre-existing TPL corepressor into new pathways as they arose during evolution, driving innovation. This hypothesis is supported by experiments where RDs have been used to convert activating transcription factors into dominant repressors (Li et al., 2007).

TPL/TPRs are recruited into diverse biological processes

Hundreds of TPL/TPR-interacting factors from more than twenty transcription-related protein families have been discovered through large-scale screening (Causier et al., 2012b). These data show that throughout evolution TPL/TPR activity has been recruited into a myriad of major signalling pathways and developmental processes and is vital for normal growth and development.

Limitations of space do not allow us to describe all known processes in which TPL/TPR proteins play a role, particularly as this is a rapidly expanding field. Instead, we will focus on a selection of major developmental programs in *Arabidopsis* requiring TPL/TPR activity, summarised in Fig. 2.

Hormone signalling

Plant development is reliant on the activity of numerous phytohormones and TPL/TPRs are critical for many phytohormone signalling pathways. Interestingly, the mechanism by which these corepressors function in the transduction of different hormone signals is remarkably similar (see Fig. 3) and is typified by auxin signalling. In a seminal paper, Szemenyei et al. (2008) described the critical role of TPL in repressing auxin-responsive genes together with AUX/IAA proteins bound to activating ARFs. AUX/IAAs mediate interactions between ARFs and TPL/TPRs, allowing TPL to inhibit inappropriate expression of ARF targets in the absence of auxin. In the presence of auxin, AUX/IAAs are ubiquitinated, marking them for degradation via the 26S proteasome. AUX/IAA degradation disrupts the TPL-ARF association, resulting in ARF-mediated activation of auxin-responsive genes. All genetic components of the canonical [ARF]-[AUX/IAA]-[TPL/TPR] mechanism regulating auxin responses are conserved from Charophyte algae through to the angiosperms (Flores-Sandoval et al., 2015; Martin-Arevalillo et al., 2019), although it remains to be determined whether the mechanism is functionally conserved. However, additional non-canonical auxin signalling mechanisms that utilise TPL corepressors have been identified (Causier et al., 2012a&b; Kuhn et al., 2020). For example, the atypical ARF ETTIN (ETT) cannot recruit AUX/IAA adaptors and instead directly recruits TPLs, via a BRD repression domain, to repress auxin target genes. Remarkably, ETT also acts as an auxin receptor, and auxin binding by ETT disrupts the ETT-TPL interaction, leading to de-repression of ETT targets (Kuhn et al., 2020).

Similar [TF]-[adaptor]-[TPL/TPR] regulatory modules also function in jasmonic acid (JA) and strigolactone (SL) phytohormone signalling (Fig. 3). Responses to the stress hormone JA are mediated by JAZ adaptor proteins, which recruit TPL either directly or indirectly via the NINJA (NOVEL INTERACTOR OF JAZ) or ECAP (EAR motif-containing adaptor protein) adaptor proteins (Pauwels et al., 2010; Causier et al., 2012b; Li et al., 2020). In SL signalling, the SUPRESSOR OF MORE AXILLARY GROWTH2 1-LIKE (SMXL) adaptor proteins

(D53 in rice) couple TPL activity to unknown TFs to regulate SL-responsive genes including *BRC1*, *TCP1* and *PAP1* (Wang et al., 2020). In both cases, as in auxin signalling, intracellular hormone elicits targeted proteasomal degradation of the adaptors, relieving transcriptional repression of JA- or SL-responsive genes (Fig. 3) (Pauwels et al., 2010; Jiang et al., 2013; Li et al., 2020). Uniquely amongst TPL adaptors, SMXLs can also act independently as TFs that bind directly to the promoters of *SMXL* genes where they recruit TPL to repress their own expression (Wang et al., 2020).

Although the [TF]-[adaptor]-[TPL/TPR] complex is a well-established mechanism for transcriptional repression of hormone-responsive genes, adaptor proteins also repress hormone-responsive genes passively and independent of TPL (reviewed by Tao & Estelle, 2018). It is suggested that the [adaptor]-[TF] interaction disrupts binding of transcriptional activators, bringing rapid transient repression. Later recruitment of TPL, and the chromatin modifications this causes, subsequently leads to sustained repression (Tao & Estelle, 2018).

Gibberellic acid (GA) is another important phytohormone. GA signalling also involves TPL activity and proteasome-based degradation of regulatory components. However, the mechanism used is different to that employed in auxin, JA and SL signalling. Briefly, under low intracellular GA levels, DELLA proteins interact with the GAF1 TF, activating genes for GA biosynthesis and perception. Increased GA content is perceived by the GA-receptor GID1, which targets DELLA for proteasomal degradation. Subsequently, GAF1 associates with TPL to form a repression complex (Fukazawa et al., 2015). Thus, the DELLA-GAF1/TPL-GAF1 system fine-tunes GA levels.

Brassinosteroids regulate diverse developmental processes by activating the BZR1 (BRASSINAZOLE-RESISTANT 1) and BES1 (BRI1-EMS-SUPPRESSOR 1) TFs that interact with TPL proteins (Oh et al., 2014; Ryu et al., 2014). BRs also inhibit responses to the phytohormone abscisic acid (ABA) through repression of the major ABA signalling regulator *ABI3* (*ABSCISIC ACID INSENSITIVE 3*) by [BES1]-[TPL] (Ryu et al., 2014; Espinosa-Ruiz et al., 2017). ABA responses are also controlled by NINJA-related ABSCISIC ACID INSENSITIVE 5 Binding Proteins (AFPs) (Chang et al., 2019). Aspects of this repression are partially dependent on TPL (Lynch et al., 2017).

Embryogenesis

The apical and basal poles of the plant are established during embryogenesis and is regulated by TPL. The temperature-sensitive dominant negative *tpl-1* mutant described by Long et al.

(2002) shows defects in shoot identity, including complete conversion to a second root when embryos develop at the restrictive temperature. Later genetic studies demonstrated that the *PLT* genes, which regulate basal fate, were directly repressed by TPL in the embryonic apical pole (Smith & Long, 2010; Figure 2). Further, Smith & Long (2010) uncovered HD-ZIP III factors as master regulators of shoot fate from a second-site modifier screen on *tpl-1*, which act antagonistically to PTLs in embryo apical/basal patterning. Establishment of the root pole is known to involve auxin, which activates *PLT* gene expression. In the embryonic shoot pole, TPL acts together with the AUX/IAA protein BODENLOS to repress basal identity factors such as *PLT* (Szemenyei et al., 2008).

Meristem maintenance

Unlike animals, plant organogenesis primarily occurs post-embryonically, and is driven by stem cell-containing meristems (Kieffer et al., 2006). Homeobox transcription factors belonging to the WUSCHEL/WUSCHEL-RELATED HOMEOBOX (WUS/WOX) family play important roles in maintaining different meristem types by inhibiting differentiation (Pi et al., 2015). WUS and WOX5 both recruit TPL to mediate the repression of various genes that promote cell differentiation in shoot and root meristems, respectively (Kieffer et al., 2006; Causier et al., 2012b; Pi et al., 2015; see Fig. 2). The significance of TPL activity for WUS function was demonstrated by the failure of a truncated version of WUS, lacking its TPL interaction sequences, to complement *wus* loss-of-function mutants. Remarkably, *wus* meristem defects were partially rescued when truncated WUS was fused to TPL (Causier et al., 2012b). TPL also interacts with other members of the WUS/WOX family, including WOX2 and WOX4, and may be required for their function in embryo and vascular cambium meristems, respectively (Causier et al., 2012b; Zhang & Tadege, 2015).

Organ growth

The PEAPOD (PPD) and TEOSINTE BRANCHED1/CINCINNATA/PROLIFERATING CELL FACTORS (TCP) TFs regulate organ development and size, which are important determinants of plant yield (Li et al., 2018). PPD and TCP proteins interact with KIX (KINASE-INDUCIBLE DOMAIN INTERACTING DOMAIN) and TIE1 (TCP INTERACTOR CONTAINING EAR MOTIF PROTEIN 1) adaptor proteins respectively, to recruit TPL (Li et al., 2018; Zhang et al., 2017). Proteasomal degradation of both these adaptors via the 26S proteasome attenuates TPL-mediated repression of PPD and TCP targets, providing a nuanced mechanism for the control of organ growth (Zhang et al., 2017; Li et al., 2018 and references therein). Targeted degradation of adaptor proteins may be a general mechanism for the release of transcriptional repression mediated by TPL, rather than one that is specific to hormone signalling; however, the signal(s) that trigger KIX or TIE1 degradation are unknown, so the possibility remains that this directly involves phytohormones.

Reproduction

TPL/TPRs play important roles in various aspects of plant reproduction, from the transition to flowering to establishment of the germline, through to seed dormancy. The timing of the transition from vegetative to reproductive growth is critical for reproductive success in plants and is tightly regulated. TPL plays a key role in flowering time by controlling expression of the floral inducer *FLOWERING LOCUS T* (*FT*) through direct interaction with the TOE1 TF or indirect interaction with CONSTANS (CO) (Causier et al., 2012b; Graeff et al., 2016). Interestingly, the CO-TPL interaction is facilitated by the microProtein adaptors miP1a and miP1b (Graeff et al., 2016), or by NINJA-related AFP2 (Chang et al., 2019). AFP2 and TPL are also involved in regulating ABA metabolism during germination (Chang et al., 2019), giving these proteins two pivotal roles in the plant life cycle. *FT* and *CO* expression are controlled by the floral transition factor CDF1 and TPL (Goralogia et al., 2017). Together these findings show that precise regulation of the floral transition requires TPL/TPRs acting at multiple points in the pathway to flowering.

Once plants commit to the reproductive growth phase, TPL then acts to control flower development. Together with the floral homeotic TF APETALA2 (AP2), TPL regulates floral organ identity genes (Krogan et al., 2012). TPL also mediates the termination of the floral meristem once all floral organs have been initiated. In a feedback loop, WUS activates the stamen and carpel TF *AGAMOUS*, which in turn directly activates *KNUCKLES* (KNU). *KNU* encodes a C2H2 zinc-finger protein that interacts with TPL to repress *WUS* expression, thus disrupting stem cell maintenance at the centre of the flower after the formation of carpel primordia (Bollier et al., 2018; Fig. 2).

TPL also plays a key role in germline development. First, TPL interacts with the SPOROCYTELESS (SPL) adaptor, which is required for male and female sporogenesis, to regulate its target genes (Chen et al., 2014; Wei et al., 2015). The TPL-SPL interaction was also observed in rice, suggesting that TPL's role in sporogenesis is conserved (Ren et al., 2018). Second, TPL forms repressive complexes with DUO1-ACTIVATED ZINC FINGER1 (DAZ1) and DAZ2 to inhibit repressors of sperm cell formation and germ cell division (Borg et al., 2014).

At the end of the reproductive process, TPL also influences seed dormancy. Through interaction with ETHYLENE RESPONSE FACTOR 12, TPL negatively regulates *DELAY OF GERMINATION 1* (*DOG1*) (Li et al., 2019).

The circadian clock

The circadian clock allows plants to anticipate periodic changes in their environment and to regulate gene expression accordingly (Sanchez & Kay, 2016). PRR proteins, which are consecutively expressed from morning to evening, bind the promoters of the core clock genes *CIRCADIAN CLOCK ASSOCIATED 1* (*CCA1*) and *LATE ELONGATED HYPOCOTYL* (*LHY*) restricting their expression to near dawn to control day-night cycles. TPL interacts with PPR proteins to form a [PRR]-[TPL]-[HDAC] repressive complex, suggesting that TPL corepressors are critical components of the clock (Wang et al., 2013).

Biotic and abiotic interactions

TPL has been linked to plant stress responses through several phytohormones (see Causier et al., 2012b) and is also involved in plant responses to pathogen attack. Zhu et al. (2010) demonstrated that *Arabidopsis* plants with depleted TPL/TPR activity were more susceptible to infection due to disruption of SNC1-mediated immunity responses. Additionally, TPL is recruited by TGA family bZIP transcription factors that control development and stress responses, via ROXY adaptor proteins, to repress TGA targets (Uhrig et al., 2017).

TOPLESS predates the evolution of land plants

Many of the processes which are regulated by the TPL family of corepressors, especially those involved in the genesis of complex meristems and of reproductive structures, were critical milestones in plant evolution, but this belies the ancient origin of the family. Early genome surveys suggested that TPL genes were restricted to the Streptophyta – the branch of plants that contains the embryophytic land plants and the Charophytes – since no TPL genes were identified in the other major plant lineage, the Chlorophyte green algae (De Smet et al., 2011; Martin-Arevalillo et al., 2017) (Fig. 4A). As more genome sequences have become available, we have now identified a *TPL*-like gene in the early-diverging chlorophyte *Picocystis spp.*, which shows that *TPL* was present in the last common ancestor of the Chlorophyta and the

Streptophyta (Fig. 4A&B). Despite being separated by almost one billion years of evolution, the TPL proteins identified in these two major plant lineages have maintained the characteristic domains (as shown Fig. 1) and share striking amino acid sequence and protein 3D structure conservation, particularly within the TPD (Fig. 4C&D). In our hands, homology-based searches in non-plant taxa across the tree of life, from distant relatives in animals and fungi to closely related lineages (e.g. the Rhodophyta), recovered no *TPL* homologues, implying that it originated in green plants. The function of TPL outside of land plants is poorly understood, so it will be important to explore the roles of TPL proteins in these basal taxa. We may gain insight into the part that TOPLESS played in the early history of plant evolution.

In *Arabidopsis*, the five TPL/TPR proteins fall into two clades: TPL/TPR1/TPR4 (TPR4 is unique to the eudicots) and TPR2/TPR3 (Fig. 4A). Intriguingly, phylogenetic analyses also identified a fourth clade of TPL proteins that is apparently missing from *Arabidopsis* and its close relatives (Fig. 4B; Liu et al., 2019). Within this clade are rice ABERRANT SPIKELET AND PANICLE 1 (ASP1; OsTPL in Fig. 4d) and maize REL2. Unlike in *Arabidopsis* where single TPL/TPR loss-of-function mutants are aphenotypic, *asp1* and *rel2* mutants present pleiotropic developmental phenotypes akin to those seen in the dominant negative *Arabidopsis tpl-1* (Yoshida et al., 2012; Gallavotti et al., 2010). These findings suggest that while *Arabidopsis* TPL/TPRs are functionally redundant, those in the monocots might only be partially redundant. Alternatively, TPLs belonging to the clade missing from *Arabidopsis* may have unique functions. The degree to which subfunctionalisation and neofunctionalisation have occurred in the TPL/TPR family is still not clear, though some authors have reported differences between TPL/TPR homologues in terms of TF affinity (Hao et al., 2014).

TPL/TPR homologues identified in nonvascular land plants, such as the moss *P. patens* and liverwort *Marchantia polymorpha*, interact with the minimal auxin signalling systems found in those species (Causier et al., 2012b; Flores-Sandoval et al., 2016), mirroring those found in angiosperms. Loss of function of *MpTPL* causes developmental defects consistent with altered auxin signalling (Flores-Sandoval et al., 2016).

The mechanism of auxin response is less clear outside of the land plants. For example, the charophyte alga *Coleochaete orbicularis* has genes that encode TPL and proto-ARF proteins, but not archetypal AUX/IAAs (De Smet et al., 2011; Bowman et al., 2017; Martin-Arevalillo et al., 2019). Interestingly, TPL proteins have been shown to interact directly with certain ARF proteins in *Arabidopsis*, *P. patens* and the charophyte alga *Chlorokybus atmophyticus*, even in the absence of AUX/IAA adaptors (Causier et al., 2012a&b; Martin-Arevalillo et al., 2019). As discussed above, the interaction between TPL and the atypical *Arabidopsis* ARF ETT is disrupted by auxin binding to ETT (Kuhn et al., 2020). Together these findings suggest the existence of a precursor classical auxin response pathway that predates the canonical auxin response.

The identification of alternative auxin signalling pathways that depend on TPL implies that TPL, and its mode of interaction with RD-containing proteins, existed prior to the evolution of many of its modern-day interactors, and prior to many of the biochemical and developmental innovations discussed herein. As acquisition of a short RD is necessary and possibly sufficient to recruit TOPLESS, one might imagine that TPL/TPRs have been recruited repeatedly to regulate new pathways.

Outlook

Transcriptional repression is an essential component of a plant's genetic toolkit, being essential for patterning gene expression in space and time, responses to stimuli, homeostasis, and other processes. Since the unusual phenotype of *tpl-1* was reported in 2002, TPL has emerged as a corepressor with a vital role, contributing to almost every aspect of plant life by mediating transcriptional repression for a diverse range of transcription factors (Fig. 2). While we have developed an understanding of TPL's interaction with transcription factors and its influence on downstream pathways, there are outstanding questions. First, the mechanism underlying TPL-induced histone deacetylation is not fully understood. Corepressors typically associate with HDACs as parts of larger heteromeric complexes. TPL colocalises with HDACs *in planta* (Krogan et al., 2012), but the mechanistic basis of this interaction is unknown. Interaction assays have also shown that TPL associates with homologues of proteins found in animal corepressor complexes, including HDACs, the histone-binding protein MSI4, and CHROMATIN REMODELLING 4 (Causier et al., 2012b; Krogan et al., 2012; Zheng et al., 2017) and may also act as a bridge between AUX/IAA and the Mediator complex in auxin signalling (Ito et al., 2016). However, a definitive 'TOPLESS complex' remains to be defined. Secondly, we have presented TPL as a potential source of developmental innovation; however, its roles in critical processes across more diverse taxa are unknown and cannot be fully explored by studying only the earliest and latest-diverging species. TPL family genes are, for example, known to be present in lycophytes such as *Selaginella moellendorffii* (Hao et al., 2014), yet there is a dearth of experimental data regarding their importance in the developmental apomorphies of lycophytes and other early-diverging vascular plants. Such studies would shed light on the evolutionary-developmental biology of embryogenesis, meristem organisation and many other areas. Thirdly, the extent to which post-translational modifications regulate TPL function activity, and how these might be targeted to control gene expression, warrants further investigation. Finally, while there are clear mechanisms for the release of repression by [TF]-[adaptor]-[TPL] regulatory modules, less is known about how repression is lifted when TPL interacts directly with a TF. New findings suggest that binding of small molecules directly to TPL or to associated TFs might disrupt the interaction between TPL and RD-containing factors (Nagashima et al., 2019; Kuhn et al., 2020). This raises the intriguing possibility that components of corepressor complexes may act as receptors for specific signals, which attenuate TPL-mediated transcriptional repression.

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Figure Legends

Figure 1. The TOPLESS protein contains multiple domains involved in tetramerisation and repression domain binding. (A) Full length TPL protein consisting of an N-terminus TOPLESS domain (TPD) and C-terminus WD40 domains. (B) The TPD consists of three distinct domains (LisH, CTLH and CRA) that fold into a complex tertiary structure. The N176H mutation, which confers the *tpl-1* phenotype, is located within the CRA domain. (C) Once folded, the TPD has two main interaction interfaces that first allow the formation of TPD dimers of which two subsequently interact to produce tetramers (dimers of dimers). Although EAR-type repression domain binding occurs away from the multimerisation interfaces, it appears impossible to decouple them. The red star indicates the position of the arginine mutated to a histidine residue in *tpl-1* (N176H).

Figure 2. TOPLESS proteins play multiple roles throughout plant development. TOPLESS (TPL) co-repressors are involved in developmental pathways from roots to shoots to flowers, and beyond. The figure shows the factors with which TPL interacts and, where known, the genes that are regulated.

Figure 3. Hormone signalling pathways utilise strikingly similar mechanisms to control gene expression. (A) TOPLESS (TPL) corepressors act to repress inappropriate gene expression in the absence of hormone (left). Here, TPL is recruited to transcription factors (TF) bound to the promoters of hormone-responsive genes (target(s)) via an adaptor protein. When intracellular hormone levels increase (right), the hormone is bound by the F-box component of a specific E3 ubiquitin ligase SCF. The SCF complex binds the adaptor in a hormone-dependent manner, resulting in polyubiquitination of the adaptor polypeptide and its degradation via the 26S proteasome. The TPL corepressor becomes disassociated from the target gene, promoting its expression. (B) A similar [TF]-[adaptor]-[TPL] regulatory module is employed in auxin (top), jasmonic acid (JA; middle) and strigolactone (SL; bottom} signalling. Auxin is perceived by the SCF$^{TIR/AFB}$ complex, targeting degradation of the AUX/IAA adaptor. In the case of JA, the hormone binds the SCF^{COI1} complex, targeting the JAZ adaptor for degradation. However, not all JAZ proteins have an obvious TPL-binding repression domain (hatched box). In these cases, TPL is indirectly recruited to the [TF]-[JAZ] complex by additional repression domain-containing adaptors, such as NINJA or ECAP.

Repression of SL target gene expression is relieved following SL-dependent degradation of SMXL/D53 by the SCF$^{\text{MAX2}}$ complex. (C) Non-canonical TPL-mediated hormone signalling pathways. Top: ETTIN (ETT) is an atypical ARF that binds to auxin-responsive genes and together with TPL represses expression under low auxin conditions (left). Auxin binds to ETT, disrupting its interaction with TPL, promoting target gene expression (right). Importantly, de-repression of [ETT]-[TPL] targets does not involve targeted protein degradation. Bottom: In addition to functioning as adaptor proteins (see B), SMXL proteins also function as SL-responsive transcription factors. In the absence of SL, SMXL proteins bind directly to the promoters of *SMXL* genes, where they recruit TPL to repress their own transcription. As in (B), the presence of SL promotes SMXL degradation, inducing *SMXL* expression.

Figure 4. The TOPLESS family of proteins is conserved across the streptophytes. (A) Summary of relationships among major lineages of green plants. (B) Bayesian phylogeny of TOPLESS (TPL) and TOPLESS-RELATED (TPR) proteins in species representing algae, basal land plants, monocots and eudicots. In land plants, three major clades emerge. The first (red box) clusters TOPLESS proteins from basal land plants with direct homologues in rosids (*Vitis vinifera*) and asterids (*Solanum lycopersicum*), indicating substantial conservation over a period of some 450 MY. *Arabidopsis thaliana* is not represented, highlighting a lineage-specific loss. The other two clades are specific to higher plants. They contain *Arabidopsis thaliana* proteins (underlined) TPL, TPR1 and TPR4, and TPR2 and TPR3 (green and blue boxes, respectively). The size of these clades reflects a recent, rapid expansion of the family. Phylogeny calculated with MrBayes 3.2.2 (Huelsenbeck and Ronquist, 2001). Node values indicate posterior probabilities. Key to species and accession numbers: Ps *Picocystis spp.* PsTPL QYZS01000150.1; Kn *Klebsormidium nitens* KnTPL DF237830; Pp *Physcomitrium patens* PpTPL1 XP_024396534.1, PpTPL2 XP_024384332.1; Mp *Marchantia polymorpha* MpTPL KP877967; Os *Oryza sativa* OsTPL Q0J7U6.1, OsTPR1 Q10NY2.1, OsTPR2 Q5NBT9.1; Zm *Zea mays* ZmREL2 ADM15671.1; Vv *Vitis vinifera* VvTPL1 GSVIVT01017487001, VvTPL2 GSVIVT01015571001, VvTPL3 GSVIVT01017343001, VvTPL4 GSVIVT01024440001, VvTPL5 GSVIVT01031186001, VvTPL6 GSVIVT01035940001; Sl *Solanum lycopersicum* SLTPL1 SOLYC03G117360, SlTPL2 SOLYC08G076030, SlTPL3 SOLYC01G100050, SlTPL4 SOLYC03G116750, SlTPL5 SOLYC07G008040, SlTPL6 SOLYC08G029050; At *Arabidopsis thaliana* AtTPL AT1G15750, AtTPR1 AT1G80490, AtTPR2 AT3G16830, AtTPR3 AT5G27030, AtTPR4 .

(C) The TOPLESS Domains of *Picocystis* TPL (PsTPL) and rice OsTPR2 are conserved at the sequence level. Key residues within the hydrophobic groove which are required for RD binding (Martin-Arevalillo et al., 2017) are conserved, as is the N176 residue which is mutated in *tpl-1* (green arrowhead). Identical residues are highlighted in red. Red text indicates similarity. Blue boxes surround contiguous similar/identical residues White bars underline residues forming alpha helices in the OsTPR2 structure (Ke et al., 2015). Alignment rendered with EsPRIPT (Robert and Gouet, 2014). (D) Modelling of the PsTPL TPD predicts structural similarity to OsTPR2. The hydrophobic groove involved in TF binding (red arrowhead) and the positions and orientations of nine alpha helices are largely conserved. Minor differences are notable between α7 and α8, orientated away from the hydrophobic groove, and in α8 and α9. N176 (mutated in *tpl-1*) is marked with a green arrowhead. Modelling performed using Phyre2 (intensive) (Kelley et al., 2015), querying with PsTPL truncated to n204. Querying with full-length PsTPL returns matches with twin beta propellers, which TPL proteins are predicted to form at their C termini (Liu et al., 2019).

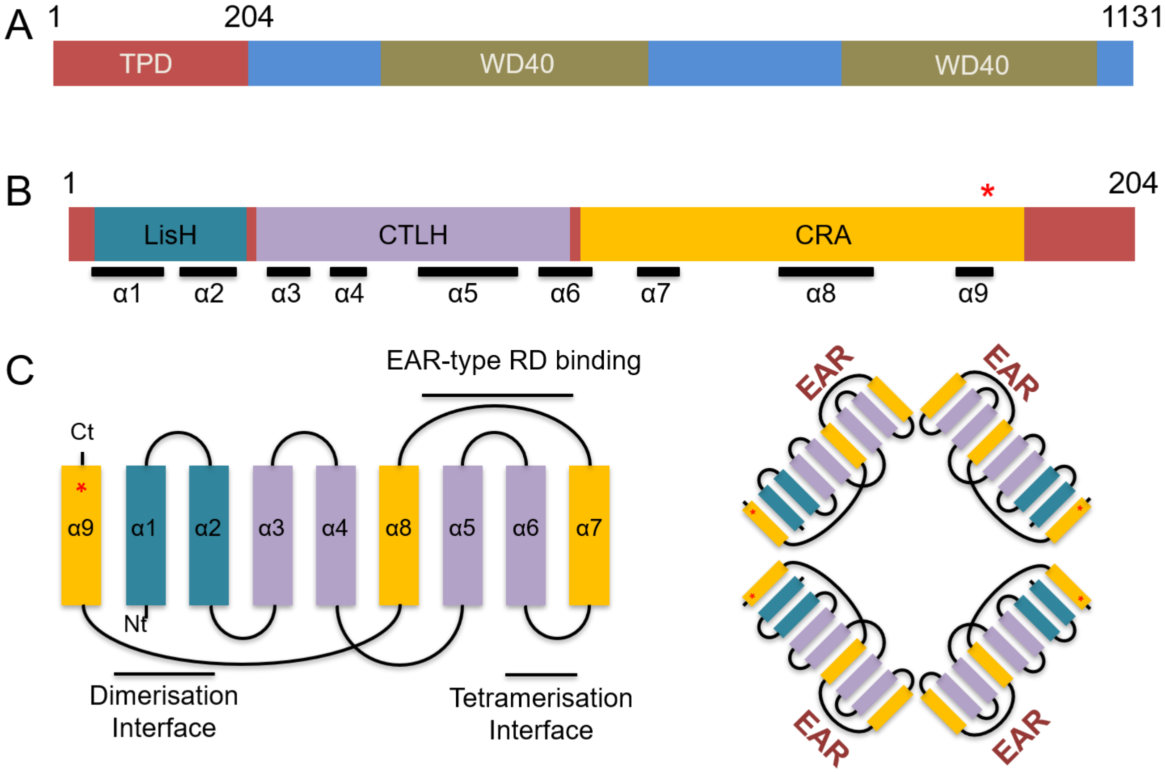


FIGURE 1

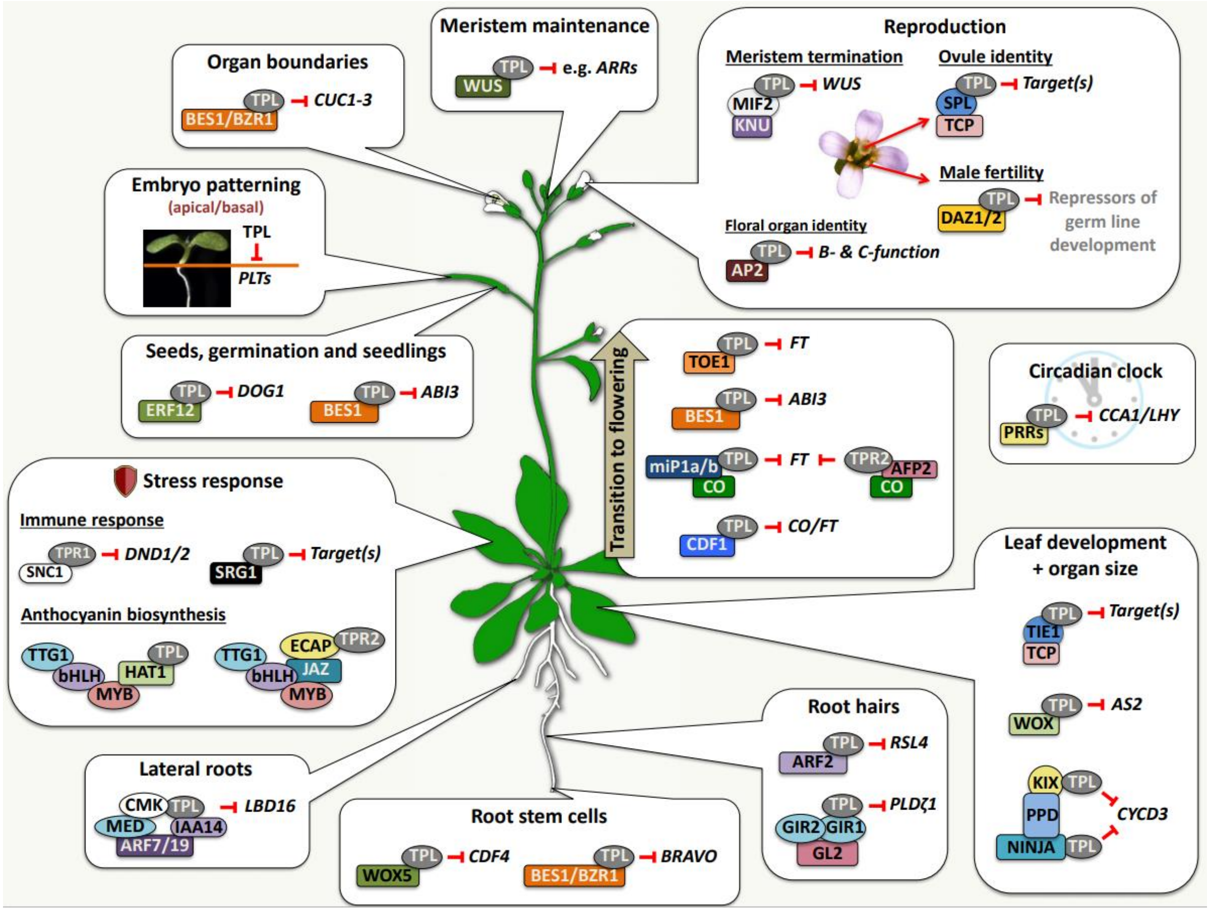
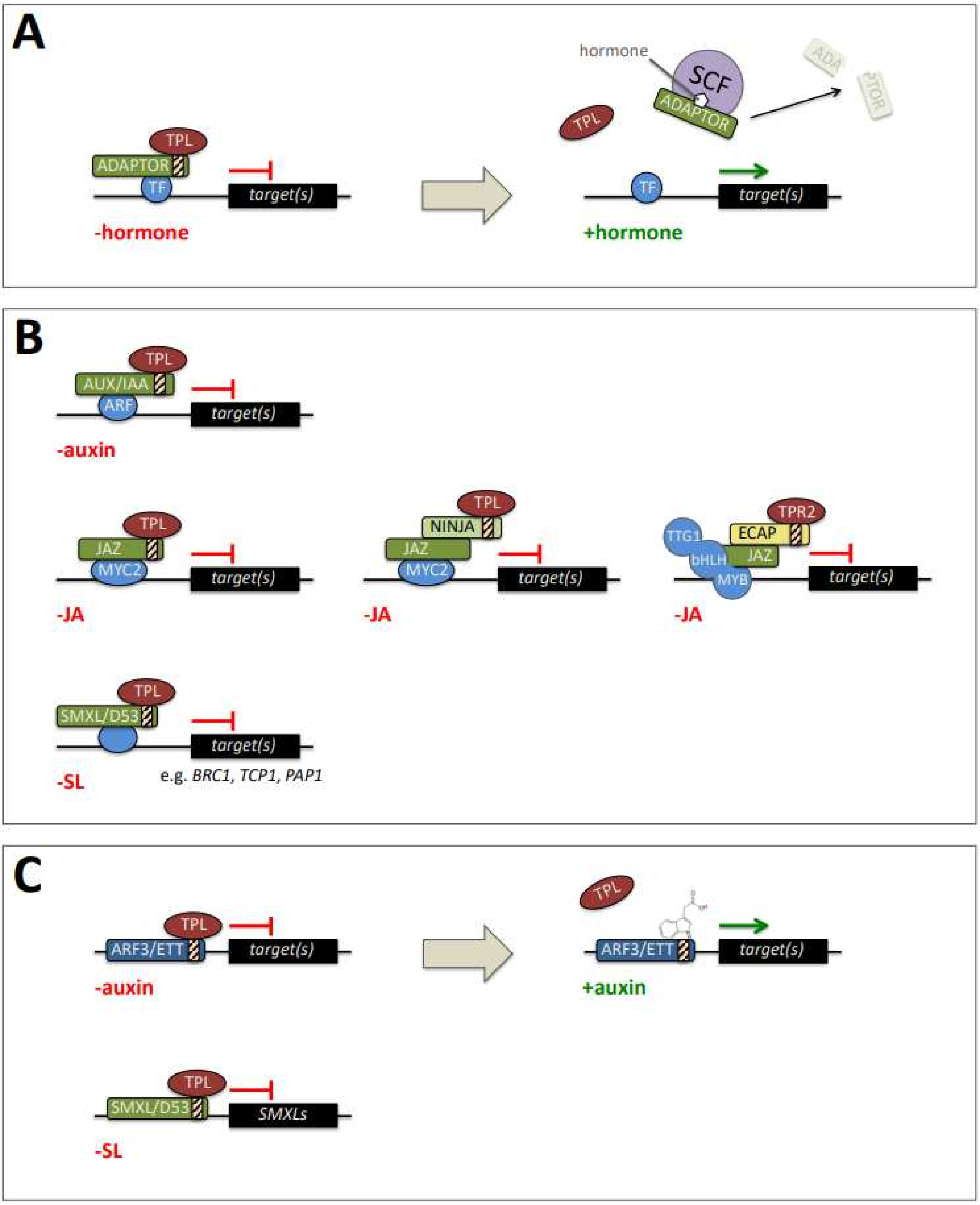

Organ boundaries
TPL
BES1/BZR1
CUC1-3
Meristem maintenance
TPL
WUS
e.g. ARRs
Reproduction
Meristem termination
TPL
MIF2
KNU
WUS
Ovule identity
TPL
SPL
TCP
Target(s)
Male fertility
TPL
DAZ1/2
Repressors of germ line development
Floral organ identity
TPL
AP2
B- & C-function
Embryo patterning
(apical/basal)
TPL
PLTs
Seeds, germination and seedlings
TPL
ERF12
DOG1
TPL
BES1
ABI3
Transition to flowering
TPL
TOE1
FT
TPL
BES1
ABI3
TPL
miP1a/b
CO
FT
TPR2
AFP2
CO
TPL
CDF1
CO/FT
Circadian clock
TPL
PRRs
CCA1/LHY
Stress response
Immune response
TPR1
SNC1
DND1/2
TPL
SRG1
Target(s)
Anthocyanin biosynthesis
TTG1
bHLH
MYB
TPL
HAT1
TTG1
bHLH
MYB
ECAP
TPR2
JAZ
Leaf development + organ size
TPL
TIE1
TCP
Target(s)
TPL
WOX
AS2
KIX
TPL
PPD
NINJA
TPL
CYCD3
Root hairs
TPL
ARF2
RSL4
TPL
GIR2
GIR1
GL2
PLDζ1
Lateral roots
CMK
TPL
MED
IAA14
ARF7/19
LBD16
Root stem cells
TPL
WOX5
CDF4
TPL
BES1/BZR1
BRAVO

FIGURE 2


A
TPL
ADAPTOR
TF
target(s)
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SCF
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ADAPTOR
ADA
PTOR
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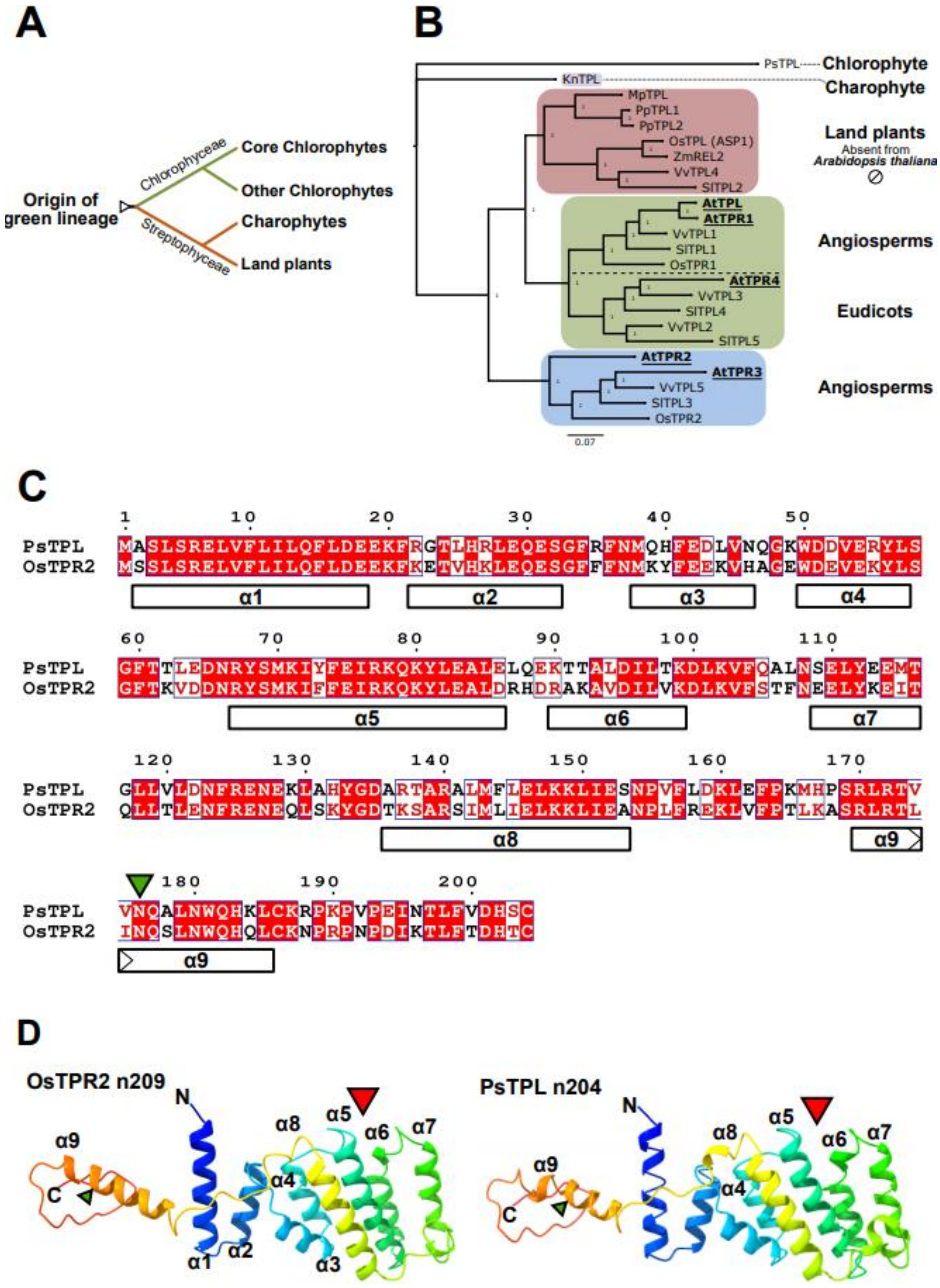
B
TPL
AUX/IAA
ARF
target(s)
-auxin
TPL
JAZ
MYC2
target(s)
-JA
TPL
NINJA
JAZ
MYC2
target(s)
-JA
TPR2
TTG1
ECAP
bHLH
JAZ
MYB
target(s)
-JA
TPL
SMXL/D53
target(s)
-SL
e.g. BRC1, TCP1, PAP1
C
TPL
ARF3/ETT
target(s)
-auxin
TPL
ARF3/ETT
target(s)
+auxin
TPL
SMXL/D53
SMXLs
-SL

FIGURE 3


A
Origin of green lineage
Chlorophyceae
Streptophyceae
Core Chlorophytes
Other Chlorophytes
Charophytes
Land plants
B
PsTPL
Chlorophyte
KnTPL
Charophyte
MpTPL
PpTPL1
PpTPL2
OsTPL (ASP1)
ZmREL2
VvTPL4
SlTPL2
Land plants
Absent from Arabidopsis thaliana
⊘
AtTPL
AtTPR1
VvTPL1
SlTPL1
OsTPR1
Angiosperms
AtTPR4
VvTPL3
SlTPL4
VvTPL2
SlTPL5
Eudicots
AtTPR2
AtTPR3
VvTPL5
SlTPL3
OsTPR2
Angiosperms
0.07
C
1 10 20 30 40 50
PsTPL MASLSRELVFLILQFLDEEKFRGTLHRLEQESGFRFNMQHFEDLVNQGKWDDVERYLS
OsTPR2 MSSLSRELVFLILQFLDEEKFKETVHKLEQESGFFFNMKYFEEKVHAGEWDEVEKYLS
α1 α2 α3 α4
60 70 80 90 100 110
PsTPL GFTTLEDNRYSMKIYFEIRKQKYLEALELQEKTTALDILTKDLKVFQALNSELYEEMT
OsTPR2 GFTKVDDNRYSMKIFFEIRKQKYLEALDRHDRAKAVDILVKDLKVFSTFNEELYKEIT
α5 α6 α7
120 130 140 150 160 170
PsTPL GLLVLDNFRENEKLAHYGDARTARALMFLELKKLIESNPVFLDKLEFPKMHPSRLRTV
OsTPR2 QLLTLENFRENEQLSKYGDTKSARSIMLIELKKLIEANPLFREKLVFPTLKASRLRTL
α8 α9
180 190 200
PsTPL VNQALNWQHKLCKRPKPVPEINTLFVDHSC
OsTPR2 INQSLNWQHQLCKNPRPNPDIKTLFTDHTC
α9
D
OsTPR2 n209
N
α9
C
α8
α5
α6
α7
α4
α1
α2
α3
PsTPL n204
N
α8
α5
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α9
α4
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FIGURE 4