Review

Nora G. Uberti Manassero, Ivana L. Viola, Elina Welchen and Daniel H. Gonzalez*

TCP transcription factors: architectures of plant form

Abstract: After its initial definition in 1999, the TCP family of transcription factors has become the focus of a multiplicity of studies related with plant development at the cellular, organ, and tissue levels. Evidence has accumulated indicating that TCP transcription factors are the main regulators of plant form and architecture and constitute a tool through which evolution shapes plant diversity. The TCP transcription factors act in a multiplicity of pathways related with cell proliferation and hormone responses. In recent years, the molecular pathways of TCP protein action and biochemical studies on their mode of interaction with DNA have begun to shed light on their mechanism of action. However, the available information is fragmented and a unifying view of TCP protein action is lacking, as well as detailed structural studies of the TCP-DNA complex. Also important, the possible role of TCP proteins as integrators of plant developmental responses to the environment has deserved little attention. In this review, we summarize the current knowledge about the structure and functions of TCP transcription factors and analyze future perspectives for the study of the role of these proteins and their use to modify plant development.

Keywords: cell proliferation and differentiation; plant architecture; plant development; TCP domain; transcription factor.

***Corresponding author**: **Daniel H. Gonzalez,** Instituto de Agrobiotecnología del Litoral (CONICET-UNL), Cátedra de Biología Celular y Molecular, Facultad de Bioquímica y Ciencias Biológicas, Universidad Nacional del Litoral, CC 242 Paraje El Pozo, 3000 Santa Fe, Argentina, e-mail: dhgonza@fbcb.unl.edu.ar

Nora G. Uberti Manassero, Ivana L. Viola and Elina Welchen: Instituto de Agrobiotecnología del Litoral (CONICET-UNL), Cátedra de Biología Celular y Molecular, Facultad de Bioquímica y Ciencias Biológicas, Universidad Nacional del Litoral, CC 242 Paraje El Pozo, 3000 Santa Fe, Argentina

Introduction

TCP proteins (TCPs) are plant transcription factors involved in diverse growth-related processes such as embryonic growth, leaf development, branching, floral organ morphogenesis, pollen development, germination, senescence, circadian rhythm, cell cycle regulation, and hormone signaling (1). These proteins contain a highly conserved domain, the TCP domain, defined by the first identified members of the family: TB1 (teosinte branched1), CYC (cycloidea), and PCF1 and 2 (2). The TCP domain is involved in DNA binding and dimerization. Secondary structure predictions suggest that the TCP domain is formed by an N-terminal region enriched in basic amino acids followed by two amphipathic α-helices connected by a disordered loop, similar to those present in bHLH eukaryotic transcription factors (Figure 1A). Sequence profile alignments between the TCP and the bHLH domains show that the hydrophobic residues involved in dimerization and interhelical interface formation in bHLH proteins are conserved in the TCP domain (3). However, the TCP basic region is longer and contains helix-breaking amino acids, which makes theoretical predictions about the nature of its contacts with DNA rather inaccurate when bHLH domain-DNA complex structures are used as templates. This defines the TCP family as a new family of transcription factors (2, 4). Evolutionary studies indicated that the TCP family arose from an unknown ancestor after the appearance of green algae and before the emergence of land plants (5).

Based on features present both within and outside the TCP domain, TCPs are divided into two classes (Figure 1B): I (also named PCF or TCP-P) and II (CYC/TB1 or TCP-C) (2, 5). Within the TCP domain, the two classes differ in three main features (Figure 1): (1) the number of residues of the basic region (class II proteins contain a 4-amino-acid insertion in this region); (2) the residue compositions in the loop and hydrophilic faces of helices I and II; (3) the length of helix II (2, 4). The class II subfamily is further divided in two lineages (Figure 1B): CIN and CYC/TB1 (or ECE). The CYC/TB1 lineage is specific

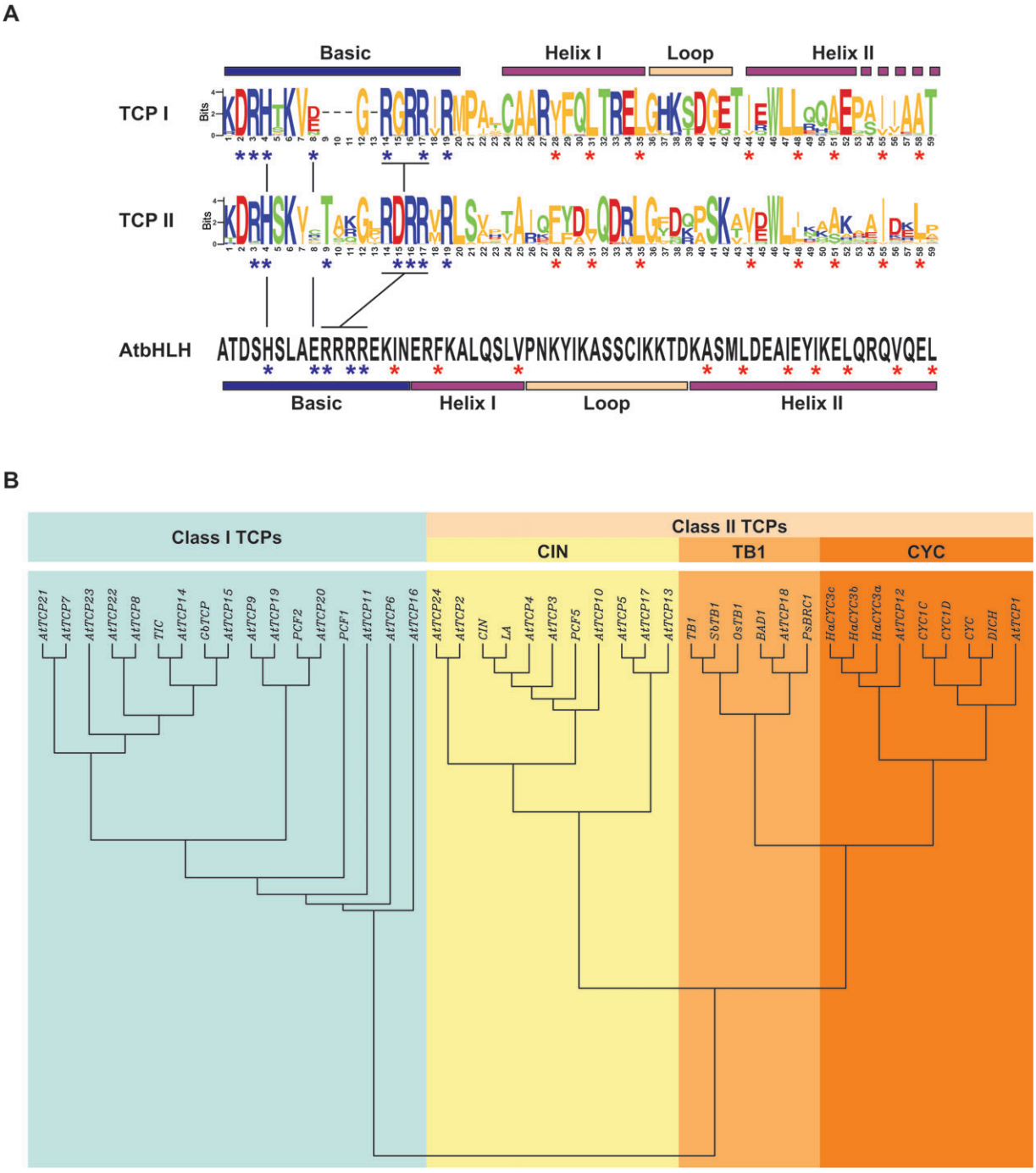

Figure 1 The TCP transcription factor family.
(A) Consensus sequences of the class I and II TCP domains. The consensus sequences of the class I and II TCP domains were derived from 244 and 108 protein sequences, respectively, using WebLogo (http://weblogo.berkeley.edu/). The putative basic, helix, and loop regions are indicated. The consensus sequence of the bHLH domain obtained by Heim et al. (99) from the 133 *Arabidopsis* bHLH proteins is shown for comparison. The residues required for DNA binding (3, 10, 11; Viola et al., unpublished data) are shown with blue asterisks; hydrophobic residues putatively involved in helix-helix interactions are shown with red asterisks. (B) Phylogenetic tree of the TCP family showing the different classes and clades mentioned in the text. The corresponding classification of the TCPs is shown in Supplementary Table S3.

of angiosperms and underwent two major duplication events just before the radiation of the core eudicots that gave rise to the CYC1, CYC2, and CYC3 clades (6). Outside the TCP domain, several class II members have an R-domain of unknown function, predicted to form a coiled coil that may mediate protein-protein interactions

(7), and class I members share short regions flanking the TCP domain.

DNA-binding properties of TCPs

Early evidence of the biochemical function of the TCP domain came from the study of class I rice PCF1 and PCF2 proteins. These proteins were isolated based on their ability to bind specifically to promoter elements of the rice proliferating cell nuclear antigen (*PCNA*) gene (4). These sequences, named site IIa (GTGGGCCCGT) and site IIb (ATGGTCCCAC, or GTGGGACCAT in the complementary strand), are essential for the proliferating cell-specific transcriptional activity of the *PCNA* gene.

A series of DNA-binding site selection assays performed with TCPs from rice (PCF2 and PCF5) (8) and *Arabidopsis* (AtTCP4, AtTCP15, and AtTCP20) (9, 10) suggested that the consensus-binding site of class I TCPs can be defined by the sequence GTGGGNCC, whereas class II proteins show a preference for the sequence GTGGNCCC. It has been established that the main determinant of the different target site preferences of class I and II TCPs is the identity of the residue present at position 11 of the basic region of the class I TCP domain or the equivalent residue 15 of the class II domain (11). Although all class II proteins contain Asp at this position, most class I proteins contain Gly (Figure 1), and reciprocal mutations produce a change in specificity. Interestingly, AtTCP16, a class I protein with Asp11, shows a preference for a class II sequence (11). It has been proposed that selection among class I and II sequences is dictated by the orientation of base-contacting amino acids, most likely arginines, located around residues 11 or 15. Subtle changes in orientation of these base contacting amino acids, brought about by interactions with other regions of the TCP domain or with other proteins, may be relevant *in vivo* for the recognition of specific target genes. The HLH motif also influences the selectivity of the basic region, allowing more or less efficient discrimination among related sequences, and determines a requirement of an extended basic region in proteins with Asp15 (11).

At present, there are no structural studies of the TCP domain or the complex that it forms with DNA. What is known about the biochemical and DNA-binding properties of the TCP domain comes from mutagenesis studies and modeling using the MyoD bHLH as template (3, 10, 11). Accordingly, it was postulated that the basic region of the TCP domain interacts with the major groove of DNA. As predicted by Cubas et al. (2), this region is mostly unstructured and the helix formation is partly induced upon DNA binding (3). AtTCP4 can form dimers both in the absence and in the presence of the target DNA, and mutations in residues predicted to be present at the dimer interface abolish DNA binding, indicating that only the dimer is able to bind DNA (3). Viola et al. (10) performed biochemical studies of the DNA-binding properties of four *Arabidopsis* class I TCPs to a 10-bp dyad-symmetric sequence composed of two GTGGG half-sites. They determined that, with the exception of AtTCP11, binding specificity relies mainly on the simultaneous interaction of both monomers with G:C base pairs located at positions 3 and 4 of both half-sites. In agreement with the consensus-binding sequences obtained for PCF2 (8), AtTCP15, and AtTCP20 (10), modifications at positions 1 and 2 of the indicated half-site produce no effect on binding as long because only one half-site is modified, suggesting that an asymmetric complex can form on DNA.

TCP target genes

Because the consensus sequences of both classes are distinct but overlapping, it has been hypothesized that TCPs from both classes share common target genes (8, 12). Nevertheless, studies using mutants and plants overexpressing native or modified forms of TCPs have suggested that partial redundancy overlaps with specific functions of different TCPs. To date, only few studies have confirmed the existence of overlapping functions between class I and II proteins. The *Arabidopsis* class I protein AtTCP15 affects the expression of genes that are also regulated by CIN class II proteins (13, 14). As described later, CIN TCPs negatively regulate the expression of *CUC* genes to promote cell differentiation in leaves (15). In the case of AtTCP3, this occurs *via* direct activation of the expression of *MIR164A*, *AS1*, *IAA3/SHY2*, and *SAUR65* (13). Interestingly, AtTCP15 acts as a regulator of two of these genes, *IAA3/SHY2* and *SAUR65*, but not the others, and this correlates with the type of target sequence present in the respective promoters (14). Recently, it has been demonstrated that *LIPOXYGENASE2* (*LOX2*), a gene involved in jasmonic acid (JA) biosynthesis, is a common target of AtTCP20 (class I) and AtTCP4 (class II), where AtTCP20 inhibits and AtTCP4 induces *LOX2* expression (9, 16). Although the hypothesis of antagonistic functions of class I and II TCPs was based on common putative target sites, AtTCP20 and AtTCP4 do not bind to the same regions of the *LOX2* promoter. This suggests that other molecular mechanisms may play a role in the antagonistic action of TCPs (16). A summary

of identified or proposed TCP target genes is presented in Supplementary Table S1.

One question that arises is whether the consensus sequences defined for each TCP class apply to all members of the respective classes or whether there are variations in DNA-binding specificities. As mentioned above, AtTCP16 prefers a class II binding site even if it belongs to class I, and this is related with the presence of Asp at position 11 of the TCP domain (11). DNA-binding selection studies suggest that changes in specificity may occur in other members of both classes. For example, the class II protein AtTCP4 selects the sequence GTGGTCCC, denoting a higher preference for T at the fifth position than PCF5 (9). Interestingly, the consensus-binding site determined for CYC, an *Antirrhinum* class II protein, is GNGGGNCC, which is more similar to a class I sequence (17). The molecular basis of this DNA-binding behavior is unknown and remains to be determined. Systematic evolution of ligands by exponential enrichment (SELEX) experiments with the class I TCPs AtTCP15 and AtTCP20 from *Arabidopsis* indicated that they have similar, but not identical, DNA-binding preferences (10). AtTCP11, another class I protein, shows a different DNA-binding behavior, with a preference for the sequence GTGGGCCN. According to mutagenesis studies, the different binding properties of AtTCP11 are attributable to the presence of Thr at position 15 of the TCP domain, which is occupied by Arg in all other *Arabidopsis* TCPs. In addition to the changes described above, the nature of the residue present at position 15 also affects the binding efficiency of class I proteins. In summary, these reports suggest that changes in DNA-binding preferences may be one of the mechanisms through which TCPs achieve functional specificity.

Protein-protein interactions

As well documented for bHLH proteins, TCP transcription factors can bind DNA as a homodimer or heterodimers. Several studies performed in rice, *Arabidopsis*, and *Primulina heterotricha* provided evidence that TCPs can form heterodimers between specific members of the same class (8, 10, 16, 18). It is interesting that the heterodimer formed by AtTCP11 and AtTCP15 binds DNA more efficiently and has a different sequence preference than the respective homodimers (10; Ripoll et al., unpublished data), suggesting that heterodimer formation may increase functional diversity of TCPs and provide a basis for specificity. In a similar manner, Danisman et al. (16) reported that only the heterodimers of AtTCP20 with AtTCP8 or AtTCP22, but not the respective homodimers, were able to interact with the *LOX2* promoter in yeast one-hybrid assays.

Several reports indicate that TCPs are also able to interact with a variety of other proteins (Supplementary Table S2). AtTCP13 interacts with the histidine-containing phosphotransmitters AHP1, AHP2, and AHP3, which have been implicated in several transduction pathways (19). AtTCP20 interacts in yeast two-hybrid assays with the transcription factor AtPuralpha, which is involved in regulating gene expression in proliferating cells (20). TIC, a class I TCP protein from *Antirrhinum*, interacts with the NAC family transcription factor CUP, involved in the formation of organ boundaries (21). The heterodimer ABAP1-AtTCP24 binds class II consensus motifs in the *AtCDT1a* and *AtCDT1b* promoters and negatively regulates their expression (22). Several *Arabidopsis* TCPs of both classes interact with different components of the core circadian clock in yeast two-hybrid and protein-protein interaction assays, indicating that the TCP family of transcription factors is linked to circadian regulation of gene expression (23, 24). In addition, there is some degree of specificity in these interactions because AtTCP21 and AtTCP3 only interact with PRR1/TOC1, AtTCP2, and AtTCP11 interact with a variety of components and AtTCP15 interacts with PRR5 (24). Furthermore, TCPs of the CIN clade (AtTCP2, AtTCP3, AtTCP4, AtTCP10, and AtTCP24) interact with ASYMMETRIC LEAVES 2 (AS2), and these complexes repress the expression of the meristem-specific class I *KNOX* genes *BP* and *KNAT2* during organ formation (25). Sugio et al. (26) found that the phytoplasma effector SAP11 interacts with a subset of CIN TCPs and mediates their destabilization, thus causing a decrease in JA synthesis. AtTCP14 and AtTCP15 interact with the *O*-GlcNAc transferase SPINDLY, and it has been proposed that these and other TCPs are covalently modified and activated as a consequence of this interaction (27). These findings suggest that the function of TCPs may be modulated through the interaction with several proteins, including a variety of non-TCP transcription factors that may provide additional specificity to TCP protein action by targeting them to specific sets of genes.

Site II elements as *cis*-regulatory element recognized by TCPs

After the identification of site IIa (GTGGGCCCGT) and site IIb (GTGGGACCAT) elements in the *PCNA* gene from rice (4), Trémousaygue et al. (20) identified similar elements (TGGGCC) in the *Arabidopsis PCNA-2* gene that are essential for expression in proliferating tissues. They

also found that such elements, thus named site II, were frequently present in the promoters of ribosomal protein genes, which are also preferentially expressed during cell proliferation, and defined the consensus sequence TGGGCY (Y=C or T) for them. The class I protein TCP20 was shown to bind these elements *in vitro* (20). Later on, site II elements were found to be present in promoters of genes involved in the transition from the G1 to S phases of the cell cycle (12), in genes expressed during initiation of axillary bud outgrowth (28) and in genes encoding components of the mitochondrial respiratory chain (29, 30). Accordingly, site II elements were proposed as coordinators of gene expression in processes related with cell growth and proliferation, like ribosomal and mitochondrial biogenesis (12, 30, 31).

Site II elements are usually present in more than one copy, located between -50 to -300 from the transcription start site (30), and are frequently associated with other *cis*-acting regulatory elements. Trémousaygue et al. (20, 32) proposed that a motif that is usually present in combination with site II elements is the telo box (internal telomeric repeat; AAACCCTAA). Telo boxes are usually located downstream of site II elements (20, 32) and operate as transcriptional enhancers of the basal activity of site II elements (20, 29, 32, 33). This association was also identified in the promoter regions of a number of genes preferentially expressed during active cell proliferation or in response to signals that promote axillary growth in *Arabidopsis* (28). Furthermore, other studies demonstrated a relationship with other regulatory elements as the tef box (ARGGRYANNNNNGT) in the promoter of the gene coding for the *Arabidopsis* translation elongation factor eEF1A, the trap40 box (GGGGGTAGAATAG) in the promoter of the gene encoding the ribosomal protein AP40, the MSA element conferring mitotic expression to the cyclin *CYCB1;1* gene (12), the G-box element (CACGTG) present in the promoter region of the cytochrome *c CYTC-2* gene (34), and initiator elements of the *COX5b-2* gene (35, 36). The general view is that combination of site II elements with other motifs confers specific transcriptional responses to different groups of genes. In addition, it has been reported that, according to the number and the architecture of site II elements in a promoter, their relative orientation, the presence of other regulatory elements, and the moment of the diurnal cycle where the genes are expressed, they can act as stimulators or repressors of gene expression (24).

There is a general consensus that TCP transcription factors are the proteins that interact with site II elements. With the exception of AtTCP8 and AtTCP22, almost all TCPs tested by Giraud et al. (24) were able to interact with site II elements in one-hybrid yeast assays. AtTCP12, AtTCP4, AtTCP17, and AtTCP21 showed the strongest interaction with both forms of site II elements, whereas AtTCP3 and AtTCP11 were more specific for TGGGCC elements and AtTCP12 preferred TGGGCT elements (24). Evidence *in vivo* has been obtained for AtTCP20, which was shown by chromatin immunoprecipitation to interact with site II-like sequences present in the promoters of six different genes (12, 16). It is noteworthy, however, that Hervé et al. (37) reported that site II elements (TGGGCY) were underrepresented in the promoters of genes downregulated in plants expressing a repressive form of AtTCP20, whereas motifs that match the class I consensus (TGGGNCC) were overrepresented. Further studies will be necessary to understand this apparent paradox and the fact that TCPs are able to interact with different sequences that, although similar, do not match the same consensus.

TCP protein functions

After initial claims that TCPs were involved in processes related with cell proliferation and growth, many studies have shed light on the specific roles of different members of the family. In a broad sense, those studies have confirmed the proposed general role of TCPs, but it also became apparent that they do so by participating in a multiplicity of processes and through different mechanisms. In the next sections, we summarize what is presently known about the functions and action mechanisms of different TCPs.

Branching

The first characterized member of the TCP family, even before the family was named by Cubas et al. in 1999 (2), was TB1, which was studied in maize by Doebley et al. (38, 39). TB1 affects the fate of maize axillary meristems: at the lower nodes, it prevents bud outgrowth, whereas at upper nodes, it promotes the development of female inflorescences (ears). In *tb1* mutants, the axillary buds of lower nodes grow out to give basal branches (tillers), and the buds of the upper nodes give branches tipped with male inflorescences (tassels), a phenotype reminiscent of the ancestor of maize, teosinte (39). Lately, Hubbard et al. (40) found that increased branching in *tb1* results from additional axillary branches formed in the axils of elongated branches. Those results supported the model proposed for the evolution of maize from teosinte, which predicts that TB1 expression would be upregulated in maize relative

to teosinte, thus resulting in the reduction of axillary branches (38).

Lateral branching is one of the most important processes that determine shoot architecture in flowering plants. Apparently, branching involves two developmental steps, bud formation and outgrowth. Since TB1 characterization, many studies relating TCP function to branching have been carried out in different species. In rice, the total number of tillers is significantly reduced by the overexpression of *OsTB1* but is increased in the *fc1* mutant containing a loss-of-function mutation of this gene. This suggests that OsTB1 functions as a negative regulator of lateral branching in rice, like maize TB1 (41). Kebrom et al. (42) demonstrated that SbTB1 (TB1 homologue from sorghum) was upregulated in plants grown under shade-avoidance conditions, thus inhibiting the development of axillary buds. The authors proposed a link between light and branching mediated by SbTB1, which is, in turn, downregulated by active phyB under favorable light conditions. BRC1 (AtTCP18) and BRC2 (AtTCP12) were identified in *Arabidopsis thaliana* as the closest homologues of TB1 by Aguilar-Martínez et al. (43). Like *tb1* mutants, *brc1* mutants showed enhanced branching, suggesting that a single ancestral mechanism for the control of branching has been maintained in distantly related angiosperm species. Similar to SbTB1, BRC1 is upregulated by plant density or shade conditions. BRC1 is also involved in two hormonal pathways that regulate axillary outgrowth, those of auxins and strigolactones (SLs). Although auxin does not seem to control BRC1 expression, BRC1 activity is necessary for the auxin-induced control of apical dominance. In addition, BRC1 is highly downregulated in loss-of-function mutants of *MAX* genes. The products of these genes promote the synthesis and activity of SLs that have been proposed to reduce auxin transport capacity in the stem, thus preventing auxin export from the buds and blocking bud outgrowth (44–46). Recently, Braun et al. (47) demonstrated that the *PsBRC1* gene from pea is mostly expressed in the axillary buds and is transcriptionally upregulated by the direct application of a synthetic SL and downregulated by cytokinins (CKs). These findings situate TB1-like TCPs as a crucial point for the determination of axillary meristem fate and the regulation of branching in angiosperms. Their action is, in turn, regulated by the balance between external (environmental) and internal (hormone) conditions to define the final architecture of plants.

Recently, a class II TCP gene named *BAD1* (branch angle defective 1) was shown to regulate the angle at which maize lateral branches emerge from the main inflorescence stem (48). BAD1, which is related in sequence to TB1, promotes cell proliferation in the pulvinus, a structure located between the stem and the lateral branches and influences lateral branch angle and plant architecture in this manner. It is noteworthy that BAD1, unlike related members of the CYC/TB1 clade, is a promoter of cell proliferation. Phylogenetic analyses suggest that orthologues of BAD1 are restricted to grasses (48), but whether their functions are the same as in maize remains to be determined.

Gametophyte development

Although TCPs from classes I and II are involved in regulating many different pathways, only a few reports mention their function in gametophyte development. Pagnussat et al. (49) first mentioned that disruption of AtTCP4 in *A. thaliana* affects early embryo development. Recently, it was observed that pollen grains from AtTCP4-VP16 plants fail to produce viable seeds, even when crossed with wild-type ovules (50). Surprisingly, pollen maturation, tube elongation, and interaction with stigmatic papillae were not affected. In addition, the carpels of AtTCP4-VP16 fertilized with wild-type pollen grains also failed to develop normal seeds. These results suggest a general reproductive dysfunction produced by the alteration of AtTCP4 expression.

Changes in pollen development were also found in plants affected in the function of two class I TCPs. Plants carrying an interference RNA for AtTCP16 showed the abortion of 50% pollen grains at early stages of development (51). The expression pattern of AtTCP16 is restricted to microspores at unicellular and bicellular stages, suggesting that AtTCP16 is involved in male gametogenesis. A similar phenotype was found in *Arabidopsis* plants overexpressing a repressive form of AtTCP11 (AtTCP11-EAR) (10). These plants produced pollen grains with an abnormal shape and their siliques were shorter than wild type and contained unfertilized ovules, probably due to the reduced number of normal pollen grains. These results indicate an involvement of TCPs in gametogenesis, although more studies are required to fully understand the role of these transcription factors in regulating plant reproduction.

Flower development

Cycloidea (CYC), another founding member of the TCP family, is responsible for the development of asymmetric flowers in *Antirrhinum* (snapdragon). *Antirrhinum* flowers are zygomorphic (asymmetric along their dorsoventral

axis), having distinct dorsal, lateral, and ventral organ types. Asymmetry is most evident in the petal and stamen whorls and depends on the action of the duplicate class II TCP genes *CYCLOIDEA* (*CYC*) and *DICHOTOMA* (*DICH*). *CYC* and *DICH* are both expressed in the dorsal domain of the floral meristem and continue to be expressed at later stages in dorsal floral organs, although the expression of *CYC* occurs in a wider region than that of *DICH* (52–54). *cyc-dich* double mutants have radially symmetric ventralized flowers, whereas single *cyc* or *dich* mutants have partially ventralized flowers, demonstrating that both proteins are necessary for the correct generation of asymmetry. Developmental analysis of single and double mutants showed that CYC and DICH can enhance or repress organ growth, depending on developmental stage and organ type, with CYC having a stronger phenotypic effect than DICH (52–54). Gaudin et al. (55) showed that the four developing stamens of *Antirrhinum* flowers expressed high levels of *CYCLIN D3b* and *HISTONE H4*, whereas the dorsal organ, the staminode, did not, suggesting that CYC/DICH function is required, either directly or indirectly, to suppress the expression of cell cycle genes.

In addition to *CYC* and *DICH*, two other genes have been shown to control dorsoventral asymmetry in *Antirrhinum*: *RADIALIS* (*RAD*) and *DIVARICATA* (*DIV*) (17, 52, 56, 57). Corley et al. (58) demonstrated that *RAD* is positively regulated by CYC and DICH. In fact, CYC is able to target class I TCP-binding sites present in the promoter and intronic regions of *RAD* (17). Similar to RAD, DIV is a MYB protein that has been proposed to be a determinant of ventral identity (17, 56). *DIV* is negatively regulated by TCPs because in *cyc-dich* double mutants, ventral identity spreads all around the flower (56).

This information allows the establishment of a model for the interactions between class II TCPs and MYB proteins to establish floral asymmetry. CYC and DICH are expressed in dorsal domains and directly upregulate RAD expression, which, in turn, antagonizes DIV function. Given the sequence similarity between RAD and DIV, this antagonism most likely could reflect molecular competition. Although not fully functional as a transcriptional activator, RAD could nevertheless inhibit DIV by competitively binding to DIV target sequences or to proteins that interact with DIV. Expression of RAD in dorsal petals would therefore prevent DIV protein from functioning in these regions. This mechanism would account for the effects of DIV being restricted to the lateral and ventral petals, even though DIV is expressed in all petals (17, 56, 58). In addition, some CYC and DICH functions may not be mediated by RAD because *rad* and *cyc-dich* mutants, although very similar, differ in several aspects (58).

The closest homologue of CYC in *A. thaliana* is AtTCP1, which was found to be transiently expressed in the dorsal parts of the floral meristem at very early stages of development (59), although its expression is not enough to generate asymmetric flowers. As *A. thaliana*, *Iberis amara* belongs to the Brassicaceae family but has a monosymmetric corolla instead of the symmetric flowers present in *Arabidopsis* plants. Adaxial petals are smaller than abaxial ones, and this fact correlates with IaTCP1 expression, which is high in the two smaller adaxial petals and low in the large abaxial ones. The overexpression of IaTCP1 in *Arabidopsis* generates narrow and small petals, although no asymmetry was found (60). As the petal cell size was similar in transgenic and wild-type plants, the expression of IaTCP1 seems to be related with inhibition of cell proliferation, as proposed for CYC in *Antirrhinum*. However, overexpression of CYC in *A. thaliana* plants led to an increase in petal size, due to an increase in cell size (17), demonstrating that CYC alters cell expansion but not proliferation in this heterologous system. A CYC-like gene from rice, *RETARDED PALEA1* (*REP1*), has been shown to influence floral zygomorphy in this species (61), suggesting the existence of common mechanisms of regulating floral asymmetry in dicot and monocot plants.

Floral zygomorphy is a key innovation associated, at least in part, with the explosive radiation of angiosperms (62). Increasing evidence indicates that *CYC/TB1* genes from the CYC2 clade were repeatedly recruited to function in the control of floral zygomorphy during evolution. Recently, Yang et al. (18) found that a double positive autoregulatory feedback loop is required for the asymmetric expression of *CYC1C* and *CYC1D*, two CYC2 clade genes from *P. heterotricha*, and that the presence of CYC-binding sites in CYC2 clade genes is positively correlated with the formation of zygomorphic flowers. This model provides a mechanism for the parallel evolution of zygomorphy in many different angiosperm lineages.

In *Gerbera hybrida*, *GhCYC2* has a symmetry function different from that of classic *CYC*-like genes. Instead of regulating the dorsoventral asymmetry of individual flowers, *GhCYC2* participates in the control of the identity of flower types in *Gerbera* composite inflorescences. The overexpression of GhCYC2 in transgenic *Gerbera* resulted in disk (inner) flowers with morphologies more similar to ray (outer) flowers. Importantly, the effect of GhCYC2 overexpression on petal growth varied among the different flower types. In ray flowers, petals were shorter (thus the entire inflorescence appeared smaller), whereas in disk flowers, petals were longer compared with wild type. Differential regulation of cell expansion and proliferation had been reported for CYC-like TCPs, but never in the same organ

type, as in this case (63). In a similar manner, a transposon insertion that inactivates the sunflower *HaCYC2c* gene generates hermaphrodite polysymmetric ray flowers, characteristics that are typical of disk flowers (64).

An interesting case is that of a natural mutant of *Linaria vulgaris* described by Linnaeus more than 250 years ago. Cubas et al. (65) found that this mutant contains flowers with radial symmetry due to the loss of expression of the *CYC* homologue *Lcyc* and that this is due to epigenetic modification of the gene.

Besides CYC, another TCP from *Antirrhinum*, CIN, has been involved in controlling cell proliferation and differentiation in petals (66). The *cin* mutants show flattened cells in petal lips, instead of the conical-shaped cells found in wild-type flowers. Opposite to CYC, CIN seems to promote cell division in petals because the mutants have smaller lips and lower expression levels of *CYCLIN D3b* and *HISTONE H4*. It is important to say that the opposite effect on cell proliferation was observed in leaves, where CIN seems to repress cell division, mostly in the leaf margins. *Arabidopsis* CIN TCPs constitute a small clade, and some of its members are negatively regulated by mir319 (67). *attcp2-3-4-10-24* loss-of-function mutants, *jaw-D plants* (which overexpress miR319) (67), or plants that express a fusion of any of the CIN TCPs to the dominant SRDX (EAR) repressor domain have similar phenotypes, with wavy petals due to excessive proliferation in the margin regions (15, 68). Accordingly, a loss of function of miR319a generated very small flowers with fused sepals and strongly repressed petals and stamens, due to ectopic expression of AtTCP4 (69). Thus, CIN TCPs seem to be responsible for repressing cell proliferation in the margin regions of the flower organs.

Recently, some class I TCPs from *Arabidopsis* have also been involved in regulating floral organ development (14, 27, 70). AtTCP14 and AtTCP15 seem to regulate the shape, size, and cell proliferation in the four whorls. The expression of dominant repressor forms of these proteins produced a decrease in the elongation of organs from the three outer whorls and the development of structures capped with stigmatic papillae from medial regions of gynoecia. AtTCP14 and AtTCP15 have also been postulated to promote CK responses mediated by the *N*-acetylglucosamine transferase SPINDLY (SPY) in flowers, possibly through direct posttranslational regulation of the TCPs by SPY (27).

Leaf development

Major efforts in revealing TCP regulatory pathways have been made with CIN class II TCPs and their effect on leaf development. CIN has been identified in *Antirrhinum* because its mutation produces leaves with altered surface curvature due to excess cell proliferation at the leaf margins (71). It was proposed that CIN activity makes cells more sensitive to a proliferation arrest signal that travels from the tip to the base of the leaf. In *Arabidopsis*, the CIN subfamily is formed by eight members (AtTCP2, 3, 4, 5, 10, 13, 17, and 24), five of them (AtTCP2, 3, 4, 10, and 24) being post-transcriptionally downregulated by miR319 (67). The high degree of functional redundancy between subfamily members has been largely demonstrated, as well as their role in regulating cell proliferation in the leaf margins. The loss of function of miR319-regulated TCPs, or the down-regulation of all members of the subfamily, generates strongly wavy and serrated leaves, demonstrating their function as negative cell proliferation factors (13, 66, 67, 71–73). Similar results were observed in plants expressing repressive forms of class II TCPs (15). According to these results, the expression of resistant forms of miR319-regulated CIN TCPs resulted in cotyledon fusion, no SAM formation, and smaller leaves (9, 17, 50, 74). Ori et al. (75) found that a gain-of-function mutation in a CIN TCP gene, *LANCEOLATE* (*LA*), produces the development of small simple leaves instead of compound leaves in tomato. Masuda et al. (22) showed that AtTCP24 is able to interact *in vivo* with AtABAP1 and AtORC1 (a protein from the pre-replication complex). The triple complex acts as a repressor of *AtCDT1a* and *b*, thus inhibiting mitotic DNA replication during leaf development. In addition, an important role of CIN TCPs as heterochronic regulators has been proposed because their spatiotemporal expression patterns seem to be highly important in controlling cell differentiation and, in this manner, leaf final shape and size (73). According to the role of CIN TCPs in promoting maturation or differentiation of cells, some authors have found that plants expressing dominant gain-of-function forms of AtTCP4 show premature senescence due, in part, to enhanced expression of JA biosynthetic enzymes (9, 50).

In the late few years, the effort has been concentrated in finding target genes of class II TCP transcription factors. In this manner, Koyama et al. (68) found that the greatest contribution to the AtTCP3-EAR phenotype is due to the increased expression of boundary-specific genes, such as *CUC* and *LOB*. *CUC* genes are negatively regulated by the AtTCP3 direct targets *AS1*, *SAUR65*, *SHY2/IAA3*, and *MIR164a* to generate the correct final shape of leaves (Figure 2). The upregulation of *CUC* genes was proposed to be responsible for the ectopic expression of class I *KNOX* genes (15) in AtTCP3-EAR plants. In addition to this indirect regulation, direct regulation of class I *KNOX* genes

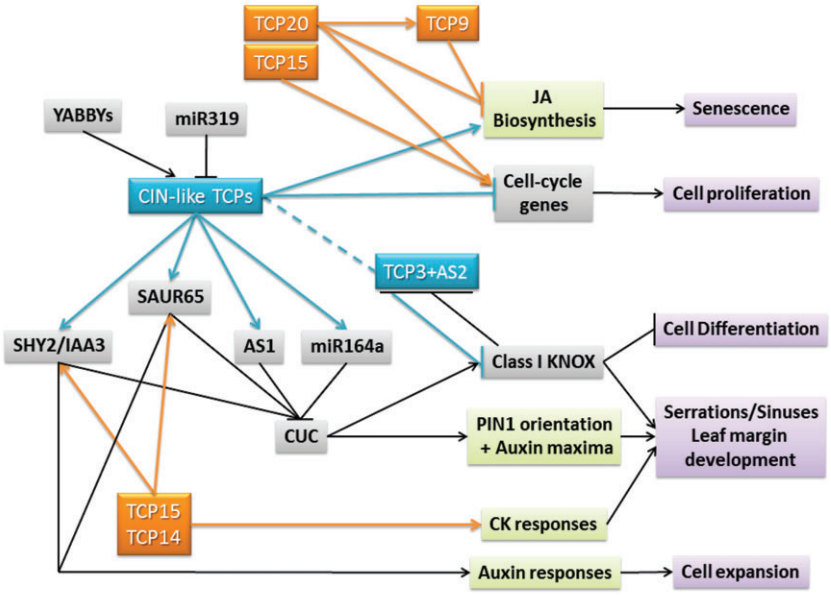

Figure 2 Molecular networks of CIN TCP protein action and their interaction with pathways modulated by class I TCPs (see text for details).

by miR319-regulated TCPs was proposed by Li et al. (25). These authors demonstrated that TCPs are able to physically interact with AS2, a known class I *KNOX* gene repressor, thus targeting the *BP/KNAT1* and *KNAT2* promoters. In this manner, miR319-regulated TCPs are able to regulate class I *KNOX* genes through both direct and indirect pathways, demonstrating again their function as repressors of cell proliferation and promoters of cell differentiation (Figure 2).

Despite the well-known post-transcriptional regulation of CIN TCPs by miR319, little is known about transcription factors that regulate them. Sarojam et al. (76) observed that *yabby* mutants fail to activate expression of CIN TCPs and, as a consequence, leaf lamina programs. More studies will be necessary to establish the nature of the interaction between YABBY proteins and CIN TCPs.

In comparison with class II TCPs, much less is known about the regulatory pathways influenced by class I proteins. Li et al. (12) showed that AtTCP20 is able to interact with the promoter region of *RIBOSOMAL PROTEIN* (*RP*) and *CYCB1;1* genes. They proposed a model in which class I TCPs mediate the marked stimulation of cell growth and division required in young lateral primordia while class II TCPs act to suppress cell growth and division as cells exit the multiplicative zone. Accordingly, organ growth would be regulated by the balance of antagonistic activities of class I and II TCPs. Nevertheless, the function of AtTCP20 in regulating organ size and development remains to be clarified because both the activator and the repressor forms of this protein generate pleiotropic (but not opposite) phenotypes in leaves, hypocotyls, and roots (37). Based on transcriptomic results and on previous studies (12, 20, 28), the authors suggested that AtTCP20 might play a role in the regulation of cell division, growth, and expansion. Recently, Danisman et al. (16) reported that single and double mutants of *AtTCP20* and *AtTCP9* show leaves with increased cell size even though total leaf area is not modified. This can be explained if AtTCP20 and AtTCP9 act to promote cell proliferation and increased cell expansion in mutants occurs as a compensatory mechanism. These authors also demonstrated that AtTCP20-AtTCP8 and AtTCP20-AtTCP22 heterodimers are able to interact with the *LOX2* promoter, a known target of class II AtTCP4. Accordingly, *LOX2* seems to be under direct antagonistic control of class II and I TCP protein complexes (Figure 2).

In the last few years, studies on three other class I TCPs have been reported. Plants that express a dominant repressive form of AtTCP11 show diminished apical dominance and altered curly leaves, although different from those produced by alteration of class II TCP function (10). Double mutants in AtTCP14 and AtTCP15 also show altered leaf development: mutant leaves are broader toward the base, have broader and shorter petioles, and their margins tend to curve upward upon maturity (70). These leaf phenotypes were considerably enhanced when AtTCP14 or AtTCP15 fusions to the EAR repressor domain were expressed in plants (14, 70). Leaves contained small epidermal and palisade cells with rounded shape, suggesting

that decreased leaf size is a consequence of reduced cell expansion not fully compensated by increased cell proliferation (14, 70). Plants expressing AtTCP14- or AtTCP15-EAR have branched trichomes (70, 77), a defect that is often associated with abnormally high ploidy due to an excessive number of endoreduplication cycles. Li et al. (77) demonstrated that TCP15-EAR induces endoreduplication in trichomes and cotyledons and, conversely, the ectopic expression of AtTCP15 causes a dramatic decrease in trichome branching and the DNA content of the nucleus. Consistently, transcript levels of *CYCA2;3* and *RBR*, negative regulators of endoreduplication, were significantly reduced in AtTCP15-EAR seedlings, whereas those of several genes that positively regulate endoreduplication were increased (77). Recently, Steiner et al. (27) proposed that both AtTCP14 and AtTCP15 promote CK responses because double mutants have leaves with smooth margins and are less sensitive to the hormone. Accordingly, the phenotype of AtTCP14-overexpressing plants is suppressed when crossed with plants with low CK levels. Besides regulating the CK responses, AtTCP15 also seems to modulate the auxin pathway by directly regulating the *SHY2/IAA3* and *SAUR* genes (14). These genes may constitute a crossing point between pathways affected by class I and II TCPs because they are also regulated by AtTCP3 (Figure 2).

Hormone pathways

A wealth of information relates TCP protein function with several hormone pathways. Aguilar-Martínez et al. (43) showed that AtBRC1/TCP18 interacts with auxin and SL pathways to modulate branching. Tomato LA represses compound leaf development by modulating gibberellin (GA) biosynthesis and degradation (78). Schommer et al. (9) showed that LOX2, a key enzyme in JA biosynthesis, is directly and positively regulated by AtTCP4. In fact, AtTCP4-VP16 plants show increased responses to GA and JA and decreased responses to auxin (50, 79). Recently, Danisman et al. (16) demonstrated that AtTCP20 also controls *LOX2* expression, antagonistically with AtTCP4. GbTCP, a class I protein from cotton closely related to AtTCP15, also influences JA biosynthesis, among other processes (80). The silencing of *GbTCP* produces plants with lower JA levels and reduced cotton fiber elongation. GbTCP also increases root hair growth initiation and elongation when overexpressed in *Arabidopsis* (80). The results suggest that different class I and II proteins have overlapping and/or antagonistic functions in the regulation of JA levels in plants.

In addition, a partially overlapping regulation was also demonstrated for class I and II AtTCP15 and AtTCP3 because the *SHY2/IAA3* and *SAUR* genes from the auxin response pathway are regulated by both proteins (13, 14). AtTCP14 and AtTCP15 seem to be activated by CK and to promote CK responses (27). AtTCP14 has also been related to abscisic acid (ABA) and GA responses during seed germination, as mentioned below (81, 82). Finally, AtTCP1 is able to directly and positively regulate the expression of DWARF4, a key enzyme in the biosynthesis of brassinosteroids (83, 84). AtTCP1 was shown to participate in the elongation of petioles, leaves, and inflorescence stems (85) and may influence these processes through the modulation of brassinosteroid homeostasis. Clearly, TCP and hormonal pathways are inextricably connected and further studies must be oriented to understand the molecular basis of these connections and how they operate to modulate plant and/or cell development.

Mitochondrial biogenesis

Mitochondrial respiratory activity is carried out by a series of multienzyme complexes consisting of subunits encoded either in the nucleus or in the same organelle. The biogenesis of respiratory complexes is a finely regulated process that requires the coordination of the expression of multiple genes (31, 86). Coordination takes place at the transcriptional level in nuclear genes, most likely through the interaction of transcription factors with common elements present in the respective promoters (30, 31). Furthermore, the expression of nuclear respiratory genes is regulated by numerous factors such as the type of tissue and developmental stage (87), nutrient availability (86, 88, 89), hormones (35), light/dark conditions (90), and the diurnal cycle (24, 91).

It has been demonstrated that the expression of mitochondrial proteins encoded by nuclear genes is controlled by site II elements, which are either responsible for basal gene expression (29, 92) or modify the magnitude of the response under different growth conditions (34, 35, 93). Over 80% of genes encoding proteins constituting complexes I, III, IV, and V of the mitochondrial respiratory machinery in *Arabidopsis* and rice have site II elements in their promoter regions (30). Site II elements act as coordinators of the response of many of these genes to several conditions, such as changes in carbohydrate availability, the activity of the photosynthetic apparatus or seed germination (31). Particularly, carbohydrate levels would operate to balance the biogenesis of the photosynthetic apparatus and the respiratory chain, which are involved in their synthesis and use, respectively.

Lee et al. (91) also showed that most genes that exhibit a strong response to changes in light/dark cycles have site II elements in their promoter regions. In contrast, genes without site II elements did not show clear cycling transcript abundance patterns. In this context, through site II element recognition, TCP transcription factors may provide a link for integrating gene expression with cellular demands for growth (24, 31).

Recently, AtTCP8 was found to interact in the nucleus with PNM1, a pentatricopeptide repeat (PPR) protein that localizes to both the nucleus and the mitochondria (94). In addition, AtTCP8 also binds to the *PNM1* promoter. As PPR proteins participate in the expression of plant mitochondrial genes, it has been postulated that the AtTCP8-PNM1 interaction may operate to adjust the expression of both genomes during mitochondrial biogenesis.

Another TCP protein that may be involved in coordinating nuclear-organellar gene expression is AtTCP13. Baba et al. (95) found that this protein, which they named PTF1, is able to interact with the promoter region of the chloroplast *psbD* gene. According to this, AtTCP13 was found to be located in chloroplasts, and *ptf1* mutants showed lower *psbD* transcript levels and pale green cotyledons. Interestingly, Suzuki et al. (19) found that AtTCP13 localizes to the nucleus in onion epidermal cell localization assays. This implies that AtTCP13 may have a dual location and may participate in the cross-regulation of the nuclear and chloroplast genomes. Further studies are required to address this point.

Seed germination

Tatematsu et al. (81) found that site II elements are overrepresented in promoters of genes that are upregulated during seed imbibition in *Arabidopsis*, suggesting a role of TCPs in this process. Accordingly, they reported that AtTCP14, which shows the highest expression level just before germination, positively modulates embryonic growth potential. AtTCP14 interacts with the transcription factor DOF6, a negative regulator of germination (82). *attcp14* mutants are hypersensitive to ABA and the GA synthesis inhibitor paclobutrazol (81), suggesting that it affects germination through the modulation of hormone responses.

Regulation of the circadian clock

The circadian clock regulates diverse aspects of plant growth and development (96). The circadian networks can be outlined as a central oscillator that generates rhythmic outputs *via* specific signaling pathways composed by positive and negative factors organized in transcriptional autoregulatory loops (97). The core clock is composed by a negative feedback regulation between the regulatory protein timing of CAB expression 1/pseudo-response regulator 1 (TOC1/PRR1) and the two MYB transcription factors circadian clock associated 1 (CCA1) and late elongated hypocotyl (LHY). Moreover, the circadian oscillator can be regulated by environmental cues such as temperature and light (96, 97). CCA1 and LHY are DNA-binding proteins that repress *TOC1* expression binding specifically to its promoter region. TOC1/PRR1, meanwhile, does not have a DNA-binding motif.

In a recent study, Pruneda-Paz et al. (23) demonstrated that the transcription factor AtTCP21 or CHE (for CCA1 hiking expedition) specifically interacts with a *CCA1* promoter fragment encompassing nucleotides -363 to -192, which contains the consensus class I TCP-binding site (GTGGGACC). In addition, AtTCP21/CHE interacts with TOC1/PRR1, which lacks a DNA-binding domain. This result allowed the authors to propose a mechanism by which TOC1/PRR1 can regulate CCA1 expression (23). Accordingly, AtTCP21 would be an important component of the circadian oscillator (23, 97). In addition, CCA1 and LHY repress *AtTCP21* expression through direct binding to a site in its promoter. A mutual regulation between CCA1 and AtTCP21 establishes a transcriptional feedback module within the *Arabidopsis* core clock network (97).

In parallel, a decrease in CCA1 expression was recently observed in plants overexpressing a translational fusion between AtTCP20 and the EAR repressor motif (37), indicating that other TCPs might be redundant with AtTCP21 for the regulation of CCA1. Because TOC1 interacts with the AtTCP21 DNA-binding domain, which is highly conserved in sequence in different members of the TCP family, it is possible that AtTCP20 and other TCP transcription factors also participate in the regulation of CCA1 expression (23).

More recently, it has been shown that other members of the TCP family (AtTCP2, AtTCP3, AtTCP11, AtTCP15) are able to interact with different regulatory proteins of the circadian clock, such as LHY, PRR1, PRR5, and CCA1 (24), and regulate the expression of several genes in accordance with power demands imposed by changes in the circadian cycle. Once again, the activity of TCP transcription factors would be related to the control of growth and development, providing a mechanism to link the circadian clock with hormonal, environmental (light, temperature, stress conditions), and nutritional/metabolic cues (97).

According to these observations, it can be postulated that TCPs have additional functions, not strictly related with the regulation of plant architecture or development (Figure 3). In addition to the regulation of the circadian

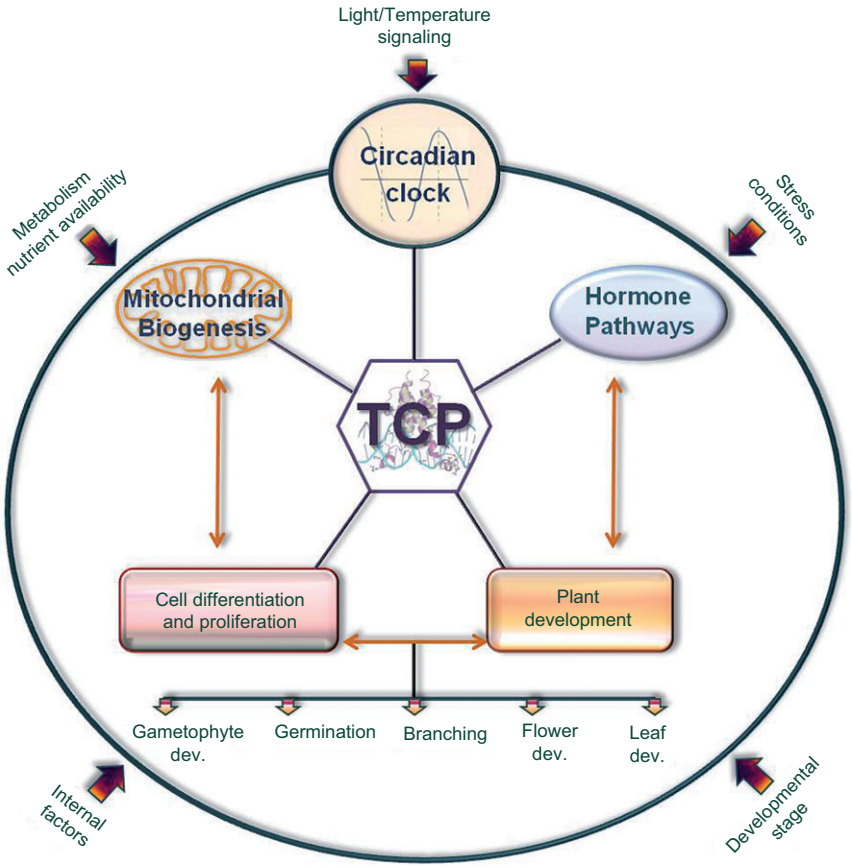

Figure 3 An integrated view of TCP protein action.
TCPs modulate plant development acting on processes related with cell growth, proliferation, and differentiation and influencing hormone pathways. Current evidence suggests that TCPs also participate in other processes, such as mitochondrial biogenesis and regulation of the circadian clock that may help to adjust plant development to internal and external factors.

cycle (23, 96), these proteins may regulate the biogenesis of ribosomes and mitochondrial respiratory complexes (12, 20, 30, 31), adjusting cell metabolism to the demands for growth and the signals received from the environment (1, 24, 31, 98).

Expert opinion

Since its definition as a new family of transcription factors, a wealth of studies has shown the importance of TCPs for plant growth and development. As outlined above, TCPs regulate many different processes at the cellular, organ, and tissue levels and integrate hormonal and developmental signals. The field is particularly exciting because it is moving toward the identification of target genes and regulatory networks affected by TCPs. This information is eagerly awaited because, for the moment, clear evidence linking molecular (either TCP-DNA or TCP-protein) interactions with phenotypic effects is not available for most of the TCPs. Also important, almost nothing is known about transcription factors that modulate TCP gene expression or other factors that are located upstream of the TCP signaling cascades.

One important question regarding TCP protein action is the degree of specificity among the many members of the family. Redundancy is evident from the study of single mutants, but there is also evidence that different members of the family perform different functions. What is the molecular basis of specificity? Does it lie within the TCP domain and the interactions it establishes with DNA and other (including TCP) proteins? Is it related with portions of the proteins that lie outside the TCP domain? Or is it a reflection of the different expression patterns of the corresponding genes? Clearly, the answer will be a combination of these (and perhaps other) possibilities, but the correct understanding of TCP protein action will require a dissection of the contribution of each of these factors.

Because the interactions established by the TCP domain are likely to be important for the function of TCPs, the three-dimensional structure of the TCP domain and its

complex with DNA will be a major advance in understanding TCP protein action. Current models based on the structure of the bHLH domain are probably highly inaccurate, mainly due to the presence of helix-breaking amino acids in the basic region of TCPs. Questions such as why a TCP homodimer binds sequences that are essentially asymmetrical and what are the residues responsible for the formation of dimers will be solved in this manner.

The way that TCPs interact with different sequences, like GTGGGNCC (class I consensus), GTGGNCCC (class II consensus), and NTGGGCY (site II element) is also a mystery. Although similar, these sequences have clearly different features as, for example, the requirement for C at position 7 in the first two, which can be replaced by T in the site II element, or the requirement of the dinucleotide GC at positions 5 and 6 of the site II element, but less strict requirements (GN or NC) in the class I and II consensus. Perhaps this reflects differences between specific TCPs or it is brought about by heterodimerization or interactions with other proteins.

Finally, the knowledge of the role of TCPs in integrating environmental signals with plant developmental responses is limited. Plants show a high degree of developmental plasticity and modify their development according to environmental conditions. Several hormonal pathways are known to mediate developmental responses to the environment, and it is likely that TCPs are important mediators as well. Studies of the molecular mechanisms of TCP protein action and of the response of plants with varying levels of TCPs to changing environmental conditions will help to elucidate this point.

Outlook

It can be envisaged that in the next few years important information about the molecular mechanisms of TCP protein action will be obtained. ChIP-Seq studies with different members of the family will help to establish the regulatory networks through which TCPs exert their action and to define consensus DNA sequences recognized by these proteins *in vivo*. Hopefully, these studies, together with structural and biochemical studies, will help to establish a molecular code for the interaction of TCPs with DNA. Interaction studies will be used to define protein-protein networks and, together with the use of native and modified forms of TCPs, the function of regions located outside the TCP domain and the molecular basis of specificity. With all this knowledge, it will be possible to use native or modified forms of TCPs, expressed in specific cell types or organs, to modulate plant growth and architecture in desirable ways. The information obtained through the comparative analysis of TCP protein function in different species and its relationship with the evolution of plant form will also be helpful for this purpose.

Highlights

- The TCP family is a novel family of transcription factors discovered in plants.
- Several studies indicate that these factors are involved in the regulation of cell growth and differentiation and, through these processes, the establishment of plant form and architecture.
- Modifications in TCP protein function have been linked to evolutionary changes in plant form diversity.
- It is becoming evident that TCPs may also link plant development with metabolic, hormonal, and environmental signals.
- The molecular mechanisms through which these proteins exert their function are beginning to be unraveled.
- Detailed knowledge of these molecular mechanisms will allow the use of these proteins to modify plant growth and form in desirable ways.

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References

1. Martín-Trillo M, Cubas P. TCP genes: a family snapshot ten years later. Trends Plant Sci 2010; 15: 31–9.
2. Cubas P, Lauter N, Doebley J, Coen E. The TCP domain: a motif found in proteins regulating plant growth and development. Plant J 1999; 18: 215–22.
3. Aggarwal P, Das Gupta M, Joseph AP, Chatterjee N, Srinivasan N, Nath U. Identification of specific DNA binding residues in the TCP family of transcription factors in Arabidopsis. Plant Cell 2010; 22: 1174–89.

4. Kosugi S, Ohashi Y. PCF1 and PCF2 specifically bind to cis elements in the rice proliferating cell nuclear antigen gene. Plant Cell 1997; 9: 1607–19.
5. Navaud O, Dabos P, Carnus E, Tremousaygue D, Hervé C. TCP transcription factors predate the emergence of land plants. J Mol Evol 2007; 65: 23–33.
6. Howarth D, Donoghue M. Phylogenetic analysis of the "ECE" (CYC/TB1) clade reveals duplications predating the core eudicots. Proc Natl Acad Sci USA 2006; 103: 9101–6.
7. Lupas A, Van Dyke M, Stock J. Predicting coil coils from protein sequences. Science 1991; 252: 1162–4.
8. Kosugi S, Ohashi Y. DNA binding and dimerization specificity and potential targets for the TCP protein family. Plant J 2002; 30: 337–48.
9. Schommer C, Palatnik JF, Aggarwal P, Chételat A, Cubas P, Farmer EE, Nath U, Weigel D. Control of jasmonate biosynthesis and senescence by miR319 targets. PLoS Biol 2008; 6: 1991–2001.
10. Viola I, Uberti Manassero N, Ripoll R, Gonzalez D. The Arabidopsis class I TCP transcription factor AtTCP11 is a developmental regulator with distinct DNA-binding properties due to the presence of a threonine residue at position 15 of the TCP domain. Biochem J 2011; 435: 143–55.
11. Viola I, Reinheimer R, Ripoll R, Uberti Manassero N, Gonzalez D. Determinants of the DNA binding specificity of class I and class II TCP transcription factors. J Biol Chem 2012; 287: 347–56.
12. Li C, Potuschak T, Colón-Carmona A, Gutiérrez RA, Doerner P. Arabidopsis TCP20 links regulation of growth and cell division control pathways. Proc Natl Acad Sci USA 2005; 102: 12978–83.
13. Koyama T, Mitsuda N, Seki M, Shinozaki K, Ohme-Takagi M. TCP transcription factors regulate the activities of ASYMMETRIC LEAVES1 and miR164, as well as the auxin response, during differentiation of leaves in Arabidopsis. Plant Cell 2010; 22: 3574–88.
14. Uberti-Manassero N, Lucero L, Viola I, Vegetti A, Gonzalez D. The class I protein AtTCP15 modulates plant development through a pathway that overlaps with the one affected by CIN-like TCP proteins. J Exp Bot 2012; 63: 809–23.
15. Koyama T, Furutani M, Tasaka M, Ohme-Takagi M. TCP transcription factors control the morphology of shoot lateral organs via negative regulation of the expression of boundary-specific genes in Arabidopsis. Plant Cell 2007; 19: 473–84.
16. Danisman S, Van Der Wal F, Dhondt S, Waites R, de Folter S, Bimbo A, van Dijk AD, Muino JM, Cutri L, Dornelas MC, Angenent GC, Immink RG. Arabidopsis class I and class II TCP transcription factors regulate jasmonic acid metabolism and leaf development antagonistically. Plant Physiol 2012; 159: 1511–23.
17. Costa MMR, Fox S, Hanna AI, Baxter C, Coen E. Evolution of regulatory interactions controlling floral asymmetry. Development 2005; 132: 5093–101.
18. Yang X, Pang HB, Liu BL, Qiu ZJ, Gao Q, Wei L, Dong Y, Wang YZ. Evolution of double positive autoregulatory feedback loops in CYCLOIDEA2 clade genes is associated with the origin of floral zygomorphy. Plant Cell 2012; 24: 1834–47.
19. Suzuki T, Sakurai K, Ueguchi C, Mizuno T. Two types of putative nuclear factors that physically interact with histidine containing phosphotransferase (Hpt) domains, signaling mediators in His-to-Asp phosphorelay, in Arabidopsis thaliana. Plant Cell Physiol 2001; 42: 37–45.
20. Trémousaygue D, Garnier L, Bardet C, Dabos P, Hervé C, Lescure B. Internal telomeric repeats and "TCP-domain" protein binding sites co-operate to regulate gene expression in Arabidopsis thaliana cycling cells. Plant J 2003; 33: 957–66.
21. Weir I, Lu J, Cook H, Causier B, Schwarz-Sommer Z, Davies B. CUPULIFORMIS establishes lateral organ boundaries in Antirrhinum. Development 2004; 131: 915–22.
22. Masuda HP, Cabral LM, De Veylder L, Tanurdzic M, de Almeida Engler J, Geelen D, Inzé D, Martienssen RA, Ferreira PC, Hemerly AS. ABAP1 is a novel plant Armadillo BTB protein involved in DNA replication and transcription. EMBO J 2008; 27: 2746–56.
23. Pruneda-Paz JL, Breton G, Para A, Kay SA. A functional genomics approach reveals CHE as a component of the Arabidopsis circadian clock. Science 2009; 323: 1481–45.
24. Giraud E, Ng S, Carrie C, Duncan O, Low J, Lee CP, Van Aken O, Millar AH, Murcha M, Whelan J. TCP transcription factors link the regulation of genes encoding mitochondrial proteins with the circadian clock in Arabidopsis thaliana. Plant Cell 2010; 22: 3921–4.
25. Li Z, Li B, Shen WH, Huang H, Dong A. TCP transcription factors interact with AS2 in the repression of class-I KNOX genes in Arabidopsis thaliana. Plant J 2012; 71: 99–107.
26. Sugio A, Kingdom HN, MacLean AM, Grieve VM, Hogenhout SA. Phytoplasma protein effector SAP11 enhances insect vector reproduction by manipulating plant development and defense hormone biosynthesis. Proc Natl Acad Sci USA 2011; 108: 1254–3.
27. Steiner E, Efroni I, Gopalraj M, Saathoff K, Tseng TS, Kieffer M, Eshed Y, Olszewski N, Weiss D. The Arabidopsis O-linked N-acetylglucosamine transferase SPINDLY interacts with class I TCPs to facilitate cytokinin responses in leaves and flowers. Plant Cell 2012; 24: 96–108.
28. Tatematsu K, Ward S, Leyser O, Kamiya Y, Nambara E. Identification of cis-elements that regulate gene expression during initiation of axillary bud outgrowth in Arabidopsis. Plant Physiol 2005; 138: 757–66.
29. Welchen E, Gonzalez DH. Differential expression of the Arabidopsis cytochrome c genes Cytc-1 and Cytc-2: evidence for the involvement of TCP-domain protein binding elements in anther- and meristem-specific expression of the Cytc-1 gene. Plant Physiol 2005; 139: 88–100.
30. Welchen E, Gonzalez DH. Overrepresentation of elements recognized by TCP-domain transcription factors in the upstream regions of nuclear genes encoding components of the mitochondrial oxidative phosphorylation machinery. Plant Physiol 2006; 141: 540–5.
31. Gonzalez DH, Welchen E, Attallah CV, Comelli RN, Mufarrege EM. Transcriptional coordination of the biogenesis of the oxidative phosphorylation machinery in plants. Plant J 2007; 51: 105–16.
32. Trémousaygue D, Manevski A, Bardet C, Lescure N, Lescure B. Plant interstitial telomere motifs participate in the control of gene expression in root meristems. Plant J 1999; 20: 553–61.
33. Manevski A, Bertoni G, Bardet C, Tremousaygue D, Lescure B. In synergy with various cis-acting elements, plant interstitial telomere motifs regulate gene expression in Arabidopsis root meristems. FEBS Lett 2000; 483: 43–6.
34. Welchen E, Viola IL, Kim HJ, Prendes LP, Comelli RN, Hong JC, Gonzalez DH. A segment containing a G-box and an ACGT motif confers differential expression characteristics and responses

to the Arabidopsis Cytc-2 gene, encoding an isoform of cytochrome c. J Exp Bot 2009; 60: 829–45.
35. Comelli R, Viola I, Gonzalez DH. Characterization of promoter elements required for expression and induction by sucrose of the Arabidopsis COX5b-1 nuclear gene, encoding the zinc-binding subunit of cytochrome c oxidase. Plant Mol Biol 2009; 69: 729–43.
36. Comelli RN, Gonzalez DH. Divergent regulatory mechanisms in the response of respiratory chain component genes to carbohydrates suggests a model for gene evolution after duplication. Plant Signal Behav 2009; 4: 1–3.
37. Hervé C, Dabos P, Bardet C, Jauneau A, Auriac MC, Ramboer A, Lacout F, Tremousaygue D. In vivo interference with AtTCP20 function induces severe plant growth alterations and deregulates the expression of many genes important for development. Plant Physiol 2009; 149: 1462–77.
38. Doebley J, Stec A, Gustus C. teosinte branched1 and the origin of maize: evidence for epistasis and the evolution of dominance. Genetics 1995; 141: 333–46.
39. Doebley J, Stec A, Hubbard L. The evolution of apical dominance in maize. Nature 1997; 86: 485–8.
40. Hubbard L, McSteen P, Doebley J, Hake S. Expression patterns and mutant phenotype of teosinte branched1 correlate with growth suppression in maize and teosinte. Genetics 2002; 162: 1927–35.
41. Takeda T, Suwa Y, Suzuki M, Kitano H, Ueguchi-Tanaka M, Ashikari M, Matsuoka M, Ueguchi C. The OsTB1 gene negatively regulates lateral branching in rice. Plant J 2003; 33: 513–20.
42. Kebrom TH, Burson BL, Finlayson SA. Phytochrome B represses teosinte branched1 expression and induces sorghum axillary bud outgrowth in response to light signals. Plant Physiol 2006; 140: 1109–17.
43. Aguilar-Martínez JA, Poza-Carrión C, Cubas P. Arabidopsis BRANCHED1 acts as an integrator of branching signals within axillary buds. Plant Cell 2007; 19: 458–72.
44. Bennett T, Sieberer T, Willett B, Booker J, Luschnig C, Leyser O. The Arabidopsis MAX pathway controls shoot branching by regulating auxin transport. Curr Biol 2006; 16: 553–63.
45. Gomez-Roldan V, Fermas S, Brewer PB, Puech-Pagès V, Dun EA, Pillot JP, Letisse F, Matusova R, Danoun S, Portais JC, Bouwmeester H, Bécard G, Beveridge CA, Rameau C, Rochange SF. Strigolactone inhibition of shoot branching. Nature 2008; 45: 189–94.
46. Umehara M, Hanada A, Yoshida S, Akiyama K, Arite T, Takeda-Kamiya N, Magome H, Kamiya Y, Shirasu K, Yoneyama K, Kyozuka J, Yamaguchi S. Inhibition of shoot branching by new terpenoid plant hormones. Nature 2008; 455: 195–200.
47. Braun N, de Saint Germain A, Pillot JP, Boutet-Mercey S, Dalmais M, Antoniadi I, Li X, Maia-Grondard A, Le Signor C, Bouteiller N, Luo D, Bendahmane A, Turnbull C, Rameau C. The pea TCP transcription factor PsBRC1 acts downstream of strigolactones to control shoot branching. Plant Physiol 2012; 158: 225–38.
48. Bai F, Reinheimer R, Durantini D, Kellogg EA, Schmidt RJ. CP transcription factor, BRANCH ANGLE DEFECTIVE 1 (BAD1), is required for normal tassel branch angle formation in maize. Proc Natl Acad Sci USA 2012; 109: 12225–30.
49. Pagnussat GC, Yu HJ, Ngo QA, Rajani S, Mayalagu S, Johnson CS, Capron A, Xie LF, Ye D, Sundaresan V. Genetic and molecular identification of genes required for female gametophyte development and function in Arabidopsis. Development 2005; 132: 603–14.
50. Sarvepalli K, Nath U. Hyper-activation of the TCP4 transcription factor in Arabidopsis thaliana accelerates multiple aspects of plant maturation. Plant J 2011; 67: 595–607.
51. Takeda T, Amano K, Ohto M, Nakamura K, Sato S, Kato T, Tabata S, Ueguchi C. RNA interference of the Arabidopsis putative transcription factor TCP16 gene results in abortion of early pollen development. Plant Mol Biol 2006; 61: 165–77.
52. Almeida J, Rocheta M, Galego L. Genetic control of flower shape in Antirrhinum majus. Development 1997; 124: 1387–92.
53. Luo D, Carpenter R, Copsey L, Vincent C, Clark J, Coen E. Control of organ asymmetry in flowers of Antirrhinum. Cell 1999; 99: 367–76.
54. Luo D, Carpenter R, Vincent C, Copsey L, Coen E. Origin of floral asymmetry in Antirrhinum. Nature 1996; 383: 794–9.
55. Gaudin V, Lunness PA, Fobert PR, Towers M, Riou-Khamlichi C, Murray JA, Coen E, Doonan JH. The expression of D-cyclin genes defines distinct developmental zones in snapdragon apical meristems and is locally regulated by the Cycloidea gene. Plant Physiol 2000; 122: 1137–48.
56. Galego L, Almeida J. Role of DIVARICATA in the control of dorsoventral asymmetry in Antirrhinum flowers. Genes Dev 2002; 16: 880–91.
57. Coen ES. Floral symmetry. EMBO J 1996; 15: 6777–88.
58. Corley SB, Carpenter R, Copsey L, Coen E. Floral asymmetry involves an interplay between TCP and MYB transcription factors in Antirrhinum. Proc Natl Acad Sci USA 2005; 102: 5068–73.
59. Cubas P, Coen E, Zapater JM. Ancient asymmetries in the evolution of flowers. Curr Biol 2001; 11: 1050–2.
60. Busch A, Zachgo S. Control of corolla monosymmetry in the Brassicaceae Iberis amara. Proc Natl Acad Sci USA 2007; 104: 16714–9.
61. Yuan Z, Gao S, Xue DW, Luo D, Li LT, Ding SY, Yao X, Wilson ZA, Qian Q, Zhang DB. RETARDED PALEA1 controls palea development and floral zygomorphy in rice. Plant Physiol 2009; 149: 235–44.
62. Preston JC, Hileman LC. Conservation and diversification of the symmetry developmental program among close relatives of snapdragon with divergent floral morphologies. New Phytol 2009; 182: 751–62.
63. Broholm SK, Tähtiharju S, Laitinen RA, Albert VA, Teeri TH, Elomaa P. A TCP domain transcription factor controls flower type specification along the radial axis of the Gerbera (Asteraceae) inflorescence. Proc Natl Acad Sci USA 2008; 105: 9117–22.
64. Fambrini M, Salvini M, Pugliesi C. A transposon-mediate inactivation of a CYCLOIDEA-like gene originates polysymmetric and androgynous ray flowers in Helianthus annuus. Genetica 2011; 139: 1521–9.
65. Cubas P, Vincent C, Coen E. An epigenetic mutation responsible for natural variation in floral symmetry. Nature 1999; 401: 157–61.
66. Crawford B, Nath U, Carpenter R, Coen E. CINCINNATA controls both cell differentiation and growth in petal lobes and leaves of Antirrhinum. Plant Physiol 2004; 135: 244–53.
67. Palatnik JF, Allen E, Wu X, Schommer C, Schwab R, Carrington JC, Weigel, D. Control of leaf morphogenesis by micro-RNAs. Nature 2003; 425: 257–63.
68. Koyama T, Ohme-Takagi M, Sato F. Generation of serrated and wavy petals by inhibition of the activity of TCP transcription

factors in Arabidopsis thaliana. Plant Signal Behav 2011; 6: 697–9.
69. Nag A, King S, Jack T. miR319a targeting of TCP4 iscritical for petal growth and development in Arabidopsis. Proc Natl Acad Sci USA 2009; 106: 22534–9.
70. Kieffer M, Master V, Waites R, Davies B. TCP14 and TCP15 affect internode length and leaf shape in Arabidopsis. Plant J 2011; 68: 147–58.
71. Nath U, Crawford BC, Carpenter R, Coen E. Genetic control of surface curvature. Science 2003; 299: 1404–7.
72. Palatnik JF, Wollmann H, Schommer C, Schwab R, Boisbouvier J, Rodriguez R, Warthmann N, Allen E, Dezulian T, Huson D, Carrington JC, Weigel D. Sequence and expression differences underlie functional specialization of Arabidopsis microRNAs miR159 and miR319. Dev Cell 2007; 13: 115–25.
73. Efroni I, Blum E, Goldshmidt A, Eshed Y. A protracted and dynamic maturation schedule underlies Arabidopsis leaf development. Plant Cell 2008; 20: 2293–306.
74. Rodriguez RE, Mecchia MA, Debernardi JM, Schommer C, Weigel D, Palatnik JF. Control of cell proliferation in Arabidopsis thaliana by microRNA miR396. Development 2010; 137: 103–12.
75. Ori N, Cohen AR, Etzioni A, Brand A, Yanai O, Shleizer S, Menda N, Amsellem Z, Efroni I, Pekker I, Alvarez JP, Blum E, Zamir D, Eshed Y. Regulation of LANCEOLATE by miR319 is required for compound-leaf development in tomato. Nat Genet 2007; 39: 787–91.
76. Sarojam R, Sappl PG, Goldshmidt A, Efroni I, Floyd SK, Eshed Y, Bowman JL. Differentiating Arabidopsis shoots from leaves by combined YABBY activities. Plant Cell 2010; 22: 2113–30.
77. Li ZY, Li B, Dong AW. The Arabidopsis transcription factor AtTCP15 regulates endoreduplication by modulating expression of key cell-cycle genes. Mol Plant 2012; 5: 270–80.
78. Yanai O, Shani E, Russ D, Ori N. Gibberellin partly mediates LANCEOLATE activity in tomato. Plant J 2011; 68: 571–82.
79. Nath U, Sarvepalli K. Interaction of TCP4-mediated growth module with phytohormones. Plant Signal Behav 2011; 6: 1440–3.
80. Hao J, Tu L, Hu H, Tan J, Deng F, Tang W, Nie Y, Zhang X. GbTCP, a cotton TCP transcription factor, confers fibre elongation and root hair development by a complex regulating system. J Exp Bot 2012; 63: 6267–81.
81. Tatematsu K, Nakabayashi K, Kamiya Y, Nambara E. Transcription factor AtTCP14 regulates embryonic growth potential during seed germination in Arabidopsis thaliana. Plant J 2008; 53: 42–52.
82. Rueda-Romero P, Barrero-Sicilia C, Gómez-Cadenas A, Carbonero P, Oñate-Sánchez L. Arabidopsis thaliana DOF6 negatively affects germination in non-after-ripened seeds and interacts with TCP14. J Exp Bot 2012; 63: 1937–49.
83. Guo Z, Fujioka S, Blancaflor EB, Miao S, Gou X, Li J. TCP1 modulates brassinosteroid biosynthesis by regulating the expression of the key biosynthetic gene DWARF4 in Arabidopsis thaliana. Plant Cell 2010; 22: 1161–73.
84. An J, Guo Z, Gou X, Li J. TCP1 positively regulates the expression of DWF4 in Arabidopsis thaliana. Plant Signal Behav 2012; 6: 1117–8.
85. Koyama T, Sato F, Ohme-Takagi M. A role of TCP1 in the longitudinal elongation of leaves in Arabidopsis. Biosci Biotechnol Biochem 2010; 74: 2145–7.
86. Giegé P, Sweetlove LJ, Cognat V, Leaver CJ. Coordination of nuclear and mitochondrial genome expression during mitochondrial biogenesis in Arabidopsis. Plant Cell 2005; 17: 1497–512.
87. Huang J, Struck F, Matzinger DF, Levings CS. Flower-enhanced expression of a nuclear-encoded mitochondrial respiratory protein is associated with changes in mitochondrion number. Plant Cell 1994; 6: 439–48.
88. Welchen E, Chan RL, Gonzalez DH. Metabolic regulation of genes encoding cytochrome c and cytochrome c oxidase subunit Vb in Arabidopsis. Plant Cell Environ 2002; 25: 1605–15.
89. Welchen E, Chan RL, Gonzalez DH. The promoter of the Arabidopsis nuclear gene COX5b-1, encoding subunit 5b of the mitochondrial cytochrome c oxidase, directs tissue-specific expression by a combination of positive and negative regulatory elements. J Exp Bot 2004; 55: 1997–2004.
90. Popov VN, Eprintsev AT, Fedorin DN, Igamberdiev AU. Succinate dehydrogenase in Arabidopsis thaliana is regulated by light via phytochrome A. FEBS Lett 2010; 584: 199–202.
91. Lee CP, Eubel H, Millar AH. Diurnal changes in mitochondrial function reveal daily optimization of light and dark respiratory metabolism in Arabidopsis. Mol Cell Proteomics 2010; 9: 2125–39
92. Attallah CV, Welchen E, Gonzalez DH. The promoters of Arabidopsis thaliana genes AtCOX17-1 and -2, encoding a copper chaperone involved in cytochrome c oxidase biogenesis, are preferentially active in roots and anthers and induced by biotic and abiotic stress. Physiol Plant 2007; 129: 123–34.
93. Mufarrege E, Curi G, Gonzalez DH. Common sets of promoter elements determine the expression characteristics of three Arabidopsis genes encoding isoforms of mitochondrial cytochrome c oxidase subunit 6b. Plant Cell Physiol 2009; 50: 1393–9.
94. Hammani K, Gobert A, Hleibieh K, Choulier L, Small I, Giegé P. An Arabidopsis dual-localized pentatricopeptide repeat protein interacts with nuclear proteins involved in gene expression regulation. Plant Cell 2011; 23: 730–40.
95. Baba K, Nakano T, Yamagishi K, Yoshida S. Involvement of a nuclear-encoded basic helix-loop-helix protein in transcription of the light-responsive promoter of psbD. Plant Physiol 2001; 125: 595–603.
96. Harmer SL. The circadian system in higher plants. Annu Rev Plant Biol 2009; 60: 357–77.
97. Pruneda-Paz JL, Kay SA. An expanding universe of circadian networks in higher plants. Trends Plant Sci 2010; 15: 259–65.
98. Millar AH, Whelan J, Soole K, Day DA. Organisation and regulation of mitochondrial respiration in plants. Annu Rev Plant Biol 2011; 62: 79–104.
99. Heim MA, Jakoby M, Werber M, Martin C, Weisshaar B, Bailey PC. The basic helix-loop-helix transcription factor family in plants: a genome-wide study of protein structure and functional diversity. Mol Biol Evol 2003; 20: 735–47.

Nora Uberti Manassero did her undergraduate studies in biotechnology at the University of Litoral, Santa Fe, Argentina. In 2008, she joined the research group of Dr. Daniel Gonzalez, where she is finishing her PhD working on the functional aspects of TCP transcription factors as a fellow of the National Research Council of Argentina.

Elina Welchen obtained her PhD in biological sciences from the University of Litoral in 2005. She works on the expression of genes that encode components of the mitochondrial respiratory chain with special emphasis on the mechanisms that coordinate mitochondrial biogenesis. Since 2007, she is a career researcher of the National Research Council of Argentina.

Ivana Viola obtained her PhD in biological sciences from the University of Litoral in 2008. Her work has been devoted to the study of protein-DNA interaction mechanisms of plant transcription factors involved in plant development. Since 2011, she is a career researcher of the National Research Council of Argentina.

Daniel H. Gonzalez holds a PhD in biochemistry from the University of Rosario, Argentina. After his postdoctoral stay in Munich and Strasbourg, he became an independent group leader as a researcher of the National Research Council of Argentina in 1995. Since 1999, he is a professor of molecular and cellular biology at the Faculty of Biochemistry and Biological Sciences, University of Litoral, Argentina.