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# TCP15 modulates cytokinin and auxin responses during gynoecium development in *Arabidopsis*

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**SUMMARY**

We studied the role of *Arabidopsis thaliana* TCP15, a member of the TEOSINTE BRANCHED1-CYCLOIDEA-PCF (TCP) transcription factor family, in gynoecium development. Plants that express *TCP15* from the 35SCaMV promoter (*35S:TCP15*) develop flowers with defects in carpel fusion and a reduced amount of stigmatic papillae. In contrast, the expression of TCP15 fused to a repressor domain from its own promoter causes the development of outgrowths topped with stigmatic papillae from the replum. *35S:TCP15* plants show lower levels of the auxin indoleacetic acid and reduced expression of the auxin reporter *DR5* and the auxin biosynthesis genes *YUCCA1* and *YUCCA4*, suggesting that TCP15 is a repressor of auxin biosynthesis. Treatment of plants with cytokinin enhances the developmental effects of expressing TCP15 or its repressor form. In addition, treatment of a knockout double mutant in *TCP15* and the related gene *TCP14* with cytokinin causes replum enlargement, increased development of outgrowths and the induction of the auxin biosynthesis genes *YUCCA1* and *YUCCA4*. A comparison of the phenotypes observed after cytokinin treatment of plants with altered expression levels of *TCP15* and auxin biosynthesis genes suggests that TCP15 modulates gynoecium development by influencing auxin homeostasis. We propose that the correct development of the different tissues of the gynoecium requires a balance between auxin levels and cytokinin responses and that TCP15 participates in a feed-back loop that helps to adjust this balance.

**INTRODUCTION**

The *Arabidopsis thaliana* gynoecium is a complex structure composed of several different tissues (Roeder and Yanofsky, 2006). Gynoecium development is initiated from two fused carpel primordia that form a hollow tubular structure. Later on, different tissues start to

differentiate. At stage 8, two longitudinal ridges located at the presumptive carpel fusion sites, the repla, give rise to structures that grow towards each other and originate the septum, from which ovule primordia will emerge. The replum and derived structures are known as medial tissues of the gyneocium (Bowman *et al.*, 1999). At more advanced stages the replum is also visible from the outside as a stripe of cells that divides the walls of the cylinder in two valves. In addition, groups of cells located between the replum and the valves constitute the valve margins, important for fruit dehiscence upon maturity (Girin *et al.*, 2009). At stage 10, the top of the gynoecium starts to close and two additional tissues, the style and the stigma, also derived from medial tissues, differentiate in this region (Bowman *et al.*, 1999).

It has been proposed that gynoecium development resembles organ development at the shoot apical meristem (Girin *et al.*, 2009). In fact, valves can be regarded as modified leaves and the replum can be regarded as a meristem, or quasi-meristem, that gives rise to differentiated structures like the septum, placental tissues, ovules, style and stigma. This is also supported by the kind of genes that are expressed in the different tissues, since typical meristem identity genes are expressed in the replum and their repression is required for valve development (Alonso-Cantabrana *et al.*, 2007). In agreement with the complexity of the process, many transcription factors from different families are required for the establishment of correct developmental and tissue differentiation programs. Hormones are also important, with a particular role of auxin in the formation of apical tissues (style and stigma; for reviews, see Ferrandiz *et al.*, 2010; Reyes-Olalde *et al.*, 2013). However, the picture of the different actors that participate in gynoecium development is not complete, and the specific role of many of the known actors is still not precisely known.

TEOSINTE BRANCHED1-CYCLOIDEA-PCF (TCP) transcription factors are a group of proteins that share the presence of a conserved DNA binding and dimerization motif known as the TCP domain (Cubas *et al.*, 1999). The TCP family is composed of 24 members in *Arabidopsis*. It has been shown that several members of this family participate in processes related with organ development and cell growth, proliferation and differentiation (Martin-Trillo and Cubas, 2010; Uberti Manassero *et al.*, 2013). There are two classes of TCP proteins that differ in structure and length of the TCP domain (Navaud et al., 2007; Martin-Trillo and Cubas, 2010). While a role of any TCP protein in gynoecium development has not been established before, it has been reported that some of these transcription factors may be involved in this process. The expression of repressor forms of the related *Arabidopsis* class I proteins TCP14 and TCP15, for example, causes the development of outgrowths topped with stigmatic papillae from apical and medial portions of the replum (Kieffer *et al.*, 2011; Uberti-Manassero *et al.*, 2012). In addition, gynoecia of these plants show altered valve development. Considering these observations, we have performed a more detailed study of the role of TCP15 in gynoecium development. Our results indicate that TCP15 and related proteins orchestrate the correct development of the different tissues of the gynoecium through their participation in auxin and cytokinin responses.

## RESULTS

### Ectopic expression of TCP15 decreases auxin levels

In order to get insight into TCP15 function, we obtained plants that express *TCP15* from the *35SCaMV* promoter (*35S:TCP15*). Several independent lines that contained leaves with increased epinasty and serration were observed (Figure S1a-c). Expression analysis indicated that these lines had about 25- to 35-fold increases in *TCP15* transcript levels relative to wild type (Figure S1d). Three independent lines were reproduced and used in this study with

similar results. The phenotype of *35S:TCP15* plants is somewhat opposite to the one reported for plants that express TCP15 fused to the EAR repressor domain under the control of the *TCP15* promoter (*P15:TCP15-EAR*), where hyponastic leaves were observed (Uberti-Manassero *et al.*, 2012). The opposite effect of expressing *TCP15* and *TCP15-EAR* was also observed at the molecular level, since *35S:TCP15* plants showed increased transcript levels of *IAA3*/*SHY2* and *SAUR65* (Figure S1e,f), two possible target genes that are down-regulated by *TCP15-EAR* (Uberti-Manassero *et al.*, 2012). This suggests that TCP15 acts as a transcriptional activator in plants, in agreement with the fact that it has a weak activator activity when expressed in yeast (Steiner *et al*., 2012).

In addition, *35S:TCP15* plants showed a decrease in the expression of the auxin reporter *DR5:GUS* (Figure S1g), which is ectopically expressed in *P15:TCP15-EAR* plants (Uberti-Manassero *et al.*, 2012). *35S:TCP15* plants contain significantly reduced levels of the major auxin indoleacetic acid (IAA) compared to wild type (Figure S1h). This is in agreement with the reduced expression of *DR5:GUS* in these plants and suggests that TCP15 is a negative regulator of auxin levels.

**TCP15 affects gynoecium and silique development**

Analysis of reproductive tissues showed that *35S:TCP15* plants contain a higher number of seeds per pod than wild type (Figure S2a,c). In addition, a portion of flowers (about 20%) showed gynoecia with apical carpel fusion defects and decreased amount of stigmatic tissue (Figure 1d,e). In extreme cases, lack of carpel fusion extended towards basal regions (Figure 1f,g). Fusion defects caused carpel bending and exposure of internal tissues (Figure 1h,i). Again, this phenotype is opposite to that of *P15:TCP15-EAR* plants, which show shorter siliques with reduced number of seeds (Figure S2a-c), and gynoecia with ectopic growths

topped with stigmatic tissue (Uberti-Manassero *et al.*, 2012; Figure 1j-l) and enlarged replum (Figure 2a). We also analyzed loss-of-function mutants in *TCP15* and the related gene *TCP14*, together with the corresponding double mutant (Kieffer *et al.*, 2011). Unlike single mutants, which did not show significant phenotypic changes compared to wild type, *tcp14 tcp15* mutants had shorter siliques with fewer seeds (Figure S2d). This is in agreement with the results obtained in *P15:TCP15-EAR* plants. However, replum and stigma development appeared normal in the double mutant, probably reflecting the existence of functional redundancy with other TCP family members.

Considering the defects observed in gynoecium development, we analyzed the expression pattern of *TCP15* in plants transformed with a *uidA* (*gus*) reporter fused to the *TCP15* promoter region (*P15:GUS*; Uberti-Manassero *et al.*, 2012; Figures 2b and S3). *P15:GUS* expression was detected mainly in valves, restricted to apical and basal portions at more advanced stages (Figure S3) and no expression was detected in the style or stigma. This is in agreement with results reported by Kieffer et al. (2011). Longitudinal and cross-sections of stained gynoecia confirmed that expression is mainly restricted to valves and indicated the existence of weak expression in the replum, but not in the septum (Figure 2b).

**TCP15 modulates the expression of genes involved in auxin homeostasis and style/stigma development**

To get insight into TCP15 action at the molecular level, we performed a microarray experiment using RNA extracted from *P15:TCP15-EAR* plants. The results indicated that 740 and 542 genes were up- and down-regulated, respectively, in *P15:TCP15-EAR* plants compared to wild type. An analysis of the processes that are modified by TCP15 action in light of the results described above indicated that TCP15 influences the expression of several

genes related to auxin homeostasis (Table S1). In addition, genes encoding several transcription factors involved in the development of gynoecium apical tissues were also induced in *P15:TCP15-EAR* plants (Table S1). Mutations in several of these genes cause a decrease in style and stigma development and defects in carpel fusion in apical parts of the gynoecium (Alvarez and Smyth, 1999; Heisler *et al.*, 2001; Kuusk *et al.*, 2002), which is coincident with the results observed in *35S:TCP15* plants.

**TCP15 affects the expression of auxin biosynthesis genes in reproductive tissues**

The phenotype of *35S:TCP15* gynoecia may be caused by defects in auxin homeostasis since the development of gynoecium apical tissues requires the existence of an auxin gradient with a maximum at the top (Nemhauser *et al.*, 2000). In agreement with this, *35S:TCP15* plants showed decreased *DR5:GUS* expression in apical portions of gynoecia (Figure 3a,b). We then analyzed the expression of selected genes involved in auxin homeostasis in flowers from *35S:TCP15* and *P15:TCP15-EAR* plants. For three genes involved in auxin biosynthesis in flowers, *YUCCA1*, *YUCCA4* and *YUCCA6* (Cheng *et al.*, 2006), we observed reduced transcript levels in *35S:TCP15* flowers and increased transcript levels in *P15:TCP15-EAR* plants (Figure 3c). The opposite behavior was observed for *IAA3*/*SHY2* and *SAUR65*, involved in auxin responses (Figure S4a,b), while no changes were evident for *PIN1* and *PIN7*, involved in auxin transport and distribution (Figure S4c,d).

We also measured transcript levels of genes that encode transcription factors involved in style/stigma development. For two genes, *SPATULA* (*SPT*) and *CRABS CLAW* (*CRC*) (Alvarez and Smyth, 1999; Heisler *et al.*, 2001) transcript levels were 2- to 3-fold higher in *P15:TCP15-EAR* plants but not significantly different from wild type in *35S:TCP15* plants (Figure S4e,f). For *STYLISH1* (*STY1*) (Kuusk *et al.*, 2002), about 2-fold up- or down-

regulation was observed in plants expressing the repressor or native forms of TCP15, respectively (Figure 3d). It has been reported that STY1 regulates auxin homeostasis and is a direct activator of *YUCCA4* (Sohlberg *et al.*, 2006; Eklund *et al.*, 2010). Then, the changes in expression of auxin biosynthesis genes observed in *35S:TCP15* and *P15:TCP15-EAR* plants may be the consequence, at least in part, of changes in *STY1* expression.

**TCP15 affects the response of gynoecium development to cytokinin treatment**

We have shown here that expression of native and repressor forms of TCP15 influences auxin homeostasis and the expression of auxin biosynthesis genes. Previous studies by Steiner *et al.* (2012) indicated that TCP15 action is also related with cytokinin homeostasis and/or signaling. We then analyzed the effect of cytokinin in gynoecium development in plants with altered TCP15 function. Initially, we assayed the effect of cytokinin treatment on *TCP15* expression. For this purpose, we treated *Arabidopsis* plants with the cytokinin 6-benzylaminopurine (BAP) and analyzed *TCP15* transcript levels by RT-qPCR. The results, shown in Figure 4a, indicate that *TCP15* is induced by BAP. A similar experiment indicated that *TCP15* transcript levels are not modified after treatment with the auxin naphthalene acetic acid (NAA; Figure 4a). Treatment of *P15:GUS* plants with BAP also caused an increase in GUS staining, suggesting that induction of *TCP15* by cytokinin operates at the transcriptional level (Figure 4b,c). Previously, Steiner *et al.* (2012) suggested that cytokinin also acts through a hormone-dependent post-translational modification that activates TCP15 and other TCP proteins. Accordingly, cytokinin would promote TCP15 action at two different levels. Steiner *et al.* (2012) also showed that cytokinin action is modified by TCP15, which behaves as an activator of cytokinin responses. In agreement with this, we observed that the expression of two type-A *Arabidopsis* response regulator genes (*ARR7* and *ARR15*), which are responsive to cytokinin, is modified in *35S:TCP15* and *P15:TCP15-EAR* flowers, where

they are induced and repressed, respectively (Figure 4d). To further corroborate this result *in planta*, we obtained plants that express *gus* under the control of the *ARR15* promoter (*PARR15:GUS* plants) and crossed a *PARR15:GUS* line with *35S:TCP15* plants. In a wild-type background, *ARR15* expression is restricted to the anthers and the style at stage 12 of gynoecium development and is observed also in the gynophore at maturity (Figure 4e). After crossing this line with a *35S:TCP15* line, we observed an increase in GUS levels and an extension of the GUS staining pattern to anther filaments and sepals (Figure 4f), which is in agreement with the RT-qPCR results.

We then assayed the effect of cytokinin treatment on gynoecium development. For this purpose, we sprayed plants before bolting with 100 μM BAP and then analyzed flower morphology using scanning electron microscopy (SEM). In wild-type plants, as described before (Marsch-Martinez *et al.*, 2012), BAP treatment caused the development of outgrowths, sometimes topped with stigmatic papillae, arising from either the replum or valve margins (Figure 5a-c). This phenotype is reminiscent of plants that express the repressor form of TCP15 (*P15:TCP15-EAR*; Kieffer *et al.*, 2011; Uberti-Manassero *et al.*, 2012). In *P15:TCP15-EAR* plants, BAP treatment caused an enhancement of the phenotype observed after expression of the repressor form. These plants showed altered valve development, with many constrictions and an irregular surface (Figure 5d). They also showed a marked development of laminar outgrowths covered with stigmatic papillae arising from the replum or valve margins (Figure 5d,e).

Cytokinin treatment produced different phenotypic changes in *35S:TCP15* plants. In this case, the apical part of gynoecia developed enlarged, indeterminate structures that produced carpeloid tissue (Figure 5f,h). Carpeloid tissue was also present in medial parts of

the gynoecium, arising from carpel margins (Figure 5g,i,j). The development of extra flowers from the gynophore was also observed (Figure 5g). The indeterminate outgrowths observed in the apical part of the gynoecium also contained sepaloid and petaloid tissues topped with stigmatic papillae (Figure 5k,l) and ovule-like structures (Figure 5m). The results indicate that an increase in the expression of *TCP15* produces a significantly different response of gynoecia to cytokinin, as compared to the response observed in wild-type and *P15:TCP15-EAR* plants. In *35S:TCP15* plants, cytokinin treatment seems to have an undifferentiating role in gynoecium development, as judged by the occurrence of undifferentiated tissue and extra flowers in these plants.

Single and double *tcp14* and *tcp15* mutants showed replum enlargement and outgrowths emerging from the replum or valve margins after cytokinin treatment (Figure 6a-d). This type of response is similar to that of wild-type and *P15:TCP15-EAR* plants. However, in the *tcp15* single mutant and the *tcp14 tcp15* double mutant the effect of cytokinin was more pronounced than in wild type plants (Figure 6a-d). Particularly, the replum was considerably enlarged in the *tcp14 tcp15* mutant after treatment with the hormone (Figure 6d,e,I,j). In addition, patches of cells with different characteristics than those typical of valve tissue were observed in valves of *tcp14 tcp15* treated plants (Figure 6f,g). These patches were devoid of stomata, unlike valve tissue, and their cells were arranged longitudinally in parallel stripes, resembling cells in the replum. Thus, loss of TCP15 and TCP14 function causes an altered response of gynoecia to cytokinin treatment.

Taken together, the results obtained with *35S:TCP15*, *P15:TCP15-EAR* and *tcp14 tcp15* plants indicate that TCP15 modifies the response of gynoecium tissues to cytokinin both quantitatively and qualitatively, suggesting that it affects the developmental fate of

carpel tissues after changes in cytokinin levels. The results also indicate that TCP15, and probably TCP14, act as repressors of the formation of medial tissues and inducers of longitudinal carpel growth. This is in agreement with the expression patterns of *TCP14* (Kieffer *et al.*, 2011) and *TCP15* (Figures 2b and S3; Kieffer *et al.*, 2011), which show preferential expression in valve tissues, restricted to apical and basal portions at more advanced developmental stages, but not in style or stigma, and only faintly along the replum.

**Cytokinin induces the expression of auxin biosynthesis genes in *P15:TCP15-EAR* and *tcp14 tcp15* plants**

The analysis of mutants indicated that the role of TCP15 in gynoecium development is particularly important when cytokinin levels increase. This may be due to the fact that functional redundancy with other TCP proteins is lost under these conditions. We then explored what happens with the expression of two auxin biosynthesis genes, *YUCCA1* and *YUCCA4*, whose mutation causes altered flower development (Cheng *et al.*, 2006) in plants with altered TCP15 function after cytokinin treatment. In wild-type plants, transcript levels of *YUCCA1* and *YUCCA4* were not significantly altered after BAP treatment (Figure 7a). However, in *P15:TCP15-EAR* plants the treatment caused a significant increase (2- to 3-fold with respect to untreated plants and 4- to 11-fold with respect to wild-type plants) in transcript levels of both genes (Figure 7a). The expression levels of *YUCCA1* and *YUCCA4* were correlated with the expression of the *DR5:GUS* reporter in *P15:TCP15-EAR* plants (Figure 7b). Expression of *DR5:GUS* was mainly observed in the ectopic structures of *P15:TCP15-EAR* gynoecia, which were increased after BAP treatment (Figure 7b). Notably, expression of *DR5:GUS* was not modified after BAP treatment in wild-type plants, even when the treatment caused the development of ectopic structures (Figure 7b). This is in agreement with results reported recently using *DR5:GFP* as a reporter (Zuñiga-Mayo *et al.*,

2014) and suggests that the increase in *DR5:GUS* expression is not due to the development of ectopic outgrowths but to the action of TCP15-EAR. This also agrees with the results of *YUCCA1* and *YUCCA4* expression, since transcript levels of these genes were determined shortly after BAP treatment, when developmental changes caused by the hormone were not yet evident.

Expression levels of *YUCCA1* and *YUCCA4* were not significantly different from wild-type in single *tcp14* and *tcp15* mutants. In double mutants, no changes in *YUCCA1* expression and a slight decrease (ca. 30%) of *YUCCA4* expression was observed (Figure 7c). The slight decrease in *YUCCA4* expression is unexpected in view of the results described for *P15:TCP15-EAR* plants and may be physiologically irrelevant, since *yucca4* mutants have normal gynoecia. These results may be due to functional redundancy with other TCP proteins, as suggested by the lack of phenotypic changes in gynoecia of mutant plants. Notably, *YUCCA1* and *YUCCA4* expression was significantly induced after BAP treatment in *tcp14 tcp15* plants (Figure 7c). This suggests that TCP14 and TCP15 inhibit the response of auxin biosynthesis genes to an increase in cytokinin levels. There is a correlation, then, in the phenotype of gynoecia and the expression of auxin biosynthesis genes in *tcp14 tcp15* plants, since differences with wild type in both parameters are only manifested after BAP treatment.

**Changes in the expression of auxin biosynthesis genes modify the response of gynoecia to cytokinin**

To investigate if changes in *YUCCA* gene expression correlate with the different developmental changes observed after cytokinin treatment, we analyzed double mutants in *YUCCA1* and *YUCCA4* (Cheng *et al.*, 2006) and plants that express *YUCCA1* from the 35SCaMV promoter (*35S:YUC1*; Wang *et al.*, 2011). Gynoecia of plants that overexpress

*YUCCA1* are similar to wild type (Figure 8a). However, these plants showed an overproduction of outgrowths arising from the replum and/or valve margins after cytokinin treatment (Figure 8b,c). This phenotype is reminiscent of the one observed in cytokinin-treated *tcp14 tcp15* double mutant plants. Thus, the phenotype of these plants may be in part related with the increased expression of auxin biosynthesis genes after the treatment.

In the case of *yucca1 yucca4* mutants, flowers already showed dramatic phenotypic changes under basal growth conditions (Figure 8d), as described before (Cheng *et al.*, 2006). These changes include defects in all floral organs, which were frequently absent or reduced in number in the three outer whorls. Gynoecia lacked valves, as also observed in single *yucca4* mutants treated with a polar auxin transport inhibitor (Ståldal *et al.*, 2008). Stigma was present, but usually indeterminate groups of cells were evident on the top (Figure 8d). Cytokinin treatment of these plants resulted in flowers with defects in carpel fusion and decreased amount of stigma, and the formation of ovule- and gynoecium-like structures from the inside of another gynoecium (Figure 8e; Figure S5), revealing a certain degree of indeterminacy in this region. Indeterminacy at the top of gynoecia and the production of gynoecium-like structures was also observed in *35S:TCP15* plants after BAP treatment, suggesting that reduced *YUCCA* expression may be in part responsible for the altered response of these plants to the hormone. We also analyzed the effect of BAP treatment in double hemizygous (*yucca1*/+ *yucca4*/+) plants, where gynoecium development is not severely affected. These plants showed defects in apical carpel fusion and style/stigma development after BAP treatment (Figure 8f-h). In addition, the development of outgrowths after the treatment was less pronounced than in wild-type plants and was restricted to the apical portion of the gynoecium (Figures 1j,k and 8f-h).

To effectively evaluate if changes in expression of auxin biosynthesis genes are responsible for the phenotype observed in gynoecia of plants with altered TCP15 function, we transformed *yucca1 yucca4* mutants with the construct expressing *TCP15-EAR*. Expression of *TCP15-EAR* in the *yucca1 yucca4* background caused the typical hyponastic leaf phenotype described before for *P15:TCP15-EAR* plants (Uberti-Manassero *et al.*, 2012; Figure 9a), suggesting that the effect of TCP15-EAR on leaf development is not dependent on *YUCCA1* and *YUCCA4*. In flowers, however, the *yucca* mutant phenotype predominated and the formation of ectopic stigmatic tissue was not observed (Figure 9b). In the case of *yucca1/+ yucca4/+* hemizygous plants in *P15:TCP15-EAR* background, we analyzed plants with different severity of the *P15:TCP15-EAR* phenotype. In plants with mild phenotypic changes, gynoecia appeared normal and lacked ectopic outgrowths (Figure 9c). In lines with severely affected development, valve structure was profoundly altered but the formation of ectopic outgrowths was not observed (Figure 9d). After BAP treatment, plants with milder phenotype showed irregular valve development and the presence of ectopic outgrowths (Figure 9e). However, these changes were much less pronounced than those observed in *P15:TCP15-EAR* plants in a wild-type *YUCCA* background (Figure 5d), suggesting that the changes brought about by TCP15-EAR are dependent on *YUCCA1* and *YUCCA4*.

We also crossed *35S:TCP15* plants with plants that ectopically express *YUCCA1*. Gynoecia of these plants were similar to wild type. After cytokinin treatment, the development of outgrowths from the replum was evident, but correct apical tissue development was observed (Figure 9f,g). This phenotype resembles the one observed in *35S:YUC1* plants after treatment with the hormone (Figure 8b,c), but differs from that of *35S:TCP15* plants (Figure 5f-h), suggesting that at least part of the changes in cytokinin responses observed in *35S:TCP15* plants are due to decreased auxin biosynthesis. In addition,

plants that ectopically express *YUCCA1* and *TCP15* show some characteristics of *35S:TCP15* plants, like the development of ectopic carpeloid structures and extra flowers, notably in basal parts (Figure 9f,g), indicating that TCP15 probably acts through a *YUCCA* independent pathway in these cases.

The results suggest that changes in the expression of auxin biosynthesis genes are at least in part responsible for the altered development and response to cytokinin observed in gynoecia of plants with altered TCP15 function.

## DISCUSSION

### TCP15 participates in gynoecium and silique development

Gynoecium development is a complex process that begins with the establishment of carpel primordia at early stages of flower formation (Smyth *et al.*, 1990; Roeder and Yanofsky, 2006). Later on, different tissues start to differentiate along the longitudinal and transverse axes. Correct development of apical tissues (style and stigma) is dependent on the action of several transcription factors (reviewed in Ferrandiz *et al.*, 2010). It has also been postulated that the establishment of an auxin gradient (higher in apical parts) is required for correct style and stigma development (Nemhauser *et al.*, 2000). In this work, we describe that the ectopic expression of the *Arabidopsis* TCP transcription factor TCP15 represses style and stigma development, thus producing gynoecia with decreased stigmatic tissue and/or carpel fusion defects in apical parts. This suggests that TCP15 acts as a repressor of medial tissues. A possible function of TCP15 and its close relative TCP14 as repressors of medial tissue formation is consistent with their expression patterns, since they show preferential expression in valves and almost no expression in apical parts of the gynoecium (Kieffer *et al.*, 2011). Loss of function of these genes, however, is not enough to cause the development of apical tissues in lower portions of the gynoecium, suggesting the existence of redundancy with other

genes, possibly from the *TCP* family. In agreement with this, expression of a repressor form of TCP15 from its own gene promoter produces the development of outgrowths with stigma-like structures arising from the replum. This also agrees with the fact that the *TCP15* promoter drives expression in the replum, although expression levels in this tissue are lower than those observed in valves. The repressor form of TCP15 also alters valve development, leading to the production of shorter siliques, an effect also observed in *tcp14 tcp15* double mutants.

**TCP15 influences the effect of cytokinin on gynoecium development**

Recently, Marsch-Martinez *et al.* (2012) showed that cytokinin treatment causes replum enlargement and the development of outgrowths topped with stigmatic papillae along the replum. Cytokinin treatment of *P15:TCP15-EAR* plants resulted in an increased development of outgrowths from the replum compared to wild type. This may be due to increased TCP15-EAR expression, since cytokinin treatment induces the *TCP15* promoter. In addition, it has been reported that cytokinin promotes the activation of TCP15 by post-translational modification (Steiner *et al.*, 2012). Increased expression of more active TCP15-EAR may result in an enhancement of the phenotype observed in *P15:TCP15-EAR* plants after treatment with cytokinin. In the case of *35S:TCP15* plants, cytokinin treatment produced different phenotypic changes, leading to increased dedifferentiation. This can also be explained by cytokinin-dependent activation of TCP15 action.

When applied to *tcp15* and *tcp14 tcp15* plants, cytokinin treatment resulted in increased outgrowth development and, for the double mutant, increased replum width and defects in valve development. This agrees with a role of TCP15 and TCP14 in repressing medial tissue formation. Expression of the *TCP15* promoter preferentially in valves and also

in the replum agrees with this conclusion. The fact that differences with wild type are only observed in the mutant after cytokinin treatment suggests that the role of TCP proteins in medial tissue development is particularly important under these conditions and this agrees with the reported activation of TCP proteins by cytokinin (Steiner *et al.*, 2012).

**TCP15, auxin and gynoecium development**

It has been postulated that the establishment of an auxin gradient (higher in apical parts) is required for correct style and stigma development (Nemhauser *et al.*, 2000; Larsson *et al.*, 2014; Moubayidin and Østergaard, 2014; reviewed in Sehra and Franks, 2015). Our global expression analysis suggests that TCP15 influences auxin homeostasis at multiple levels, suggesting that TCP15 may affect gynoecium development by acting on auxin homeostasis. Particularly, ectopic expression of TCP15 affects the development of apical tissues and produces a decrease in the expression of *DR5:GUS* and IAA levels, suggesting that one of the effects of TCP15 on auxin homeostasis may be related to a decrease in auxin synthesis. In agreement with this, we observed that three *YUCCA* genes show altered expression in flowers of plants that express native or repressor forms of TCP15.

While the role of auxin in stigma/style development is well established, the way in which auxin influences the development of other tissues of the gynoecium is less clear. Besides the longitudinal auxin gradient mentioned above, an auxin minimum in the valve margin and a relative maximum in the replum have been observed (Sorefan *et al.*, 2009). It is possible that optimal auxin concentrations for valve development are lower than those required for the development of medial tissues. TCP15 may contribute to valve development by maintaining these lower auxin concentrations through the repression of auxin biosynthesis genes. In addition, since auxin is involved in the development of medial tissues, the observed

defects in carpel fusion caused by the ectopic expression of *TCP15* may also be the consequence of its action on auxin homeostasis. In agreement with this, the expression of the repressor form of TCP15 produces an enlargement of medial tissues (replum and septum).

It has also been established that the correct development of apical tissues (style and stigma) is dependent on the action of several transcription factors (reviewed in Ferrandiz *et al.*, 2010). Three genes encoding transcription factors involved in style/stigma development also show increased expression in *P15:TCP15-EAR* plants. The link between CRC, SPT, and auxin action is not completely clear, but they seem to act in different, parallel, pathways (Ståldal *et al.*, 2008). In turn, STY1, a member of the SHORT INTERNODES/STYLISH family, is a direct activator of *YUCCA4* expression (Sohlberg *et al.*, 2006). Then, TCP15 may act through *STY1* to regulate auxin biosynthesis, at least in the case of *YUCCA4*. However, it is likely that neither auxin biosynthesis genes nor *STY1* are direct targets of TCP15 since they are induced by a repressor form of this protein and repressed by the native form, which most likely acts as an activator according to our data.

It is noteworthy that the hyponastic leaf phenotype observed in *P15:TCP15-EAR* plants is not affected by mutations in *YUCCA1* and *YUCCA4*. Indeed, auxin overproduction causes leaf epinasty (Zhao *et al.,* 2001), as observed in *35S:TCP15* plants, while loss-of-function of multiple *YUCCA* genes causes leaf hyponasty (Cheng *et al.,* 2006). This suggests that the leaf phenotype of plants with altered TCP15 function is not directly related to changes in auxin levels or responses.

**A role of auxin in the response of gynoecia to cytokinin**

The effect of cytokinin on gynoecium development most likely depends on auxin levels, as indicated by the different behavior of plants with altered expression of *YUCCA* genes after cytokinin treatment. Since plants with altered TCP15 function show an altered response to cytokinin and TCP15 modifies auxin homeostasis, it can be postulated that TCP15 and related proteins influence gynoecium development through the modulation of auxin homeostasis and the consequent changes in cytokinin responses. Particularly, we have focused on the role of *YUCCA* genes in the regulation of gynoecium development by TCP15, since mutations in these genes affect flower development. We observed that the response of *YUCCA1* and *YUCCA4* to cytokinin treatment is altered in *P15:TCP15-EAR* and *tcp14 tcp15* plants. In addition, the phenotypes of *35S:TCP15* and *P15:TCP15-EAR* gynoecia are modified in *35S:YUC1* and *yucca1 yucca4* backgrounds, respectively, suggesting that the action of TCP15 on gynoecium development depends on the expression levels of *YUCCA* genes and possibly on auxin levels. Nevertheless, since TCP15 seems to affect auxin homeostasis at many different levels, it is possible that genes involved in other aspects of auxin homeostasis also influence TCP15 action.

**TCP15 links cytokinin responses and auxin biosynthesis during gynoecium development**

We propose a model in which TCP15 and related proteins link cytokinin and auxin responses for correct gynoecium development in Arabidopsis (Figure 10). Cytokinin treatment produces increased replum development (Figure 10,i) and activates TCP15 (Figure 10,ii). TCP15 acts as a repressor of auxin biosynthesis genes (Figure 10,iii) and its expression pattern (higher in valves, intermediate in the replum and almost absent in style and stigma) is complementary to auxin distribution. Auxin distribution is one of the factors that contribute to correct tissue formation in the gynoecium. Higher relative auxin levels or responses were detected in apical

tissues and the replum (Girin *et al.*, 2009), suggesting that auxin is required for medial tissue formation (Figure 10,iv). In the absence of TCP15 and the related protein TCP14, cytokinin causes enhanced replum development and increased expression of *YUCCA* genes (Figure 10,v). Jones *et al.* (2010) reported that increased cytokinin levels cause a concomitant increase in auxin biosynthesis, presumably to maintain optimal levels of both hormones for correct tissue development. As judged from the effect of cytokinin on *YUCCA* gene expression in *tcp14 tcp15* mutants and *P15:TCP15-EAR* plants, TCP15 would counteract this effect of cytokinin on auxin biosynthesis. It can be proposed that TCP15 participates in a regulatory loop that links the actions of cytokinin and auxin on gynoecium development. The function of TCP15 would be to counteract the effects of cytokinin on medial tissue formation and auxin homeostasis. This is likely to be a very simplified model, since other factors probably also influence this regulatory loop. For example, it is likely that TCP15 also modulates cytokinin responses or sensitivity through a mechanism not directly related with auxin homeostasis, as reported previously in leaves and sepals (Steiner *et al.*, 2012). In addition, TCP15 and other TCP proteins seem to have a very complex role in modulating auxin homeostasis (Koyama *et al.*, 2010; Sarvepalli and Nath, 2011; Uberti-Manassero *et al.*, 2012; Li and Zachgo, 2013; Das Gupta *et al.*, 2014), and this may also influence the action of TCP15 on gynoecium development and cytokinin responses. Further studies are required to evaluate the participation of these other factors and to establish their relative importance, which will probably be different in different organs or tissues.

## EXPERIMENTAL PROCEDURES

### Plant material and growth conditions

*Arabidopsis* lines *tcp14-4*, *tcp15-3* and *DR5:GUS* (Ulmasov *et al.*, 1997) were obtained from the Arabidopsis Biological Resource Center (ABRC, Ohio State University). Double

homozygous *tcp14-4 tcp15-3* mutants (Kieffer *et al.*, 2011) were kindly provided by Dr. Simona Masiero (Universitá degli Studi di Milano, Italy). *35S:YUC1* (Wang *et al.*, 2011) and *yucca1 yucca4* mutants (Cheng *et al.*, 2006) were provided by Drs. Lin Xu (Shanghai Institute of Plant Physiology and Ecology, China) and Yunde Zhao (University of California at San Diego), respectively. *P15:TCP15-EAR* and *P15:GUS* plants were obtained in our laboratory and were described before (Uberti-Manassero *et al.*, 2012). All lines are in Col-0 background.

Plants were grown on soil at 22–24ºC under a 16 h light/8 h dark photoperiod. Illumination at an intensity of 100 µE $m^{-2}$ $s^{-1}$ was provided by cool-white and GroLux fluorescent lamps. For cytokinin treatment, plants grown on soil before bolting were sprayed 5 days per week with 100 µM BAP (Sigma-Aldrich). After two to four weeks of treatment, samples were collected for scanning electron microscopy (SEM) analysis. For gene expression analysis, samples were collected 2 h after the first cytokinin treatment.

**Gene cloning and plant transformation**

To obtain *35S:TCP15* plants, the *TCP15* coding sequence was amplified with primers T15-F and T15-R (Table S2), digested with *Xba*I/*Xho*I and cloned in pBI121. To construct *PARR15:GUS*, nucleotides –1222 to –1 from the start codon of *ARR15* were amplified from genomic DNA using primers ProARR15-F and ProARR15-R (Table S2), and cloned into vector pBI101.3 using *Hin*dIII/*Xba*I sites. After checking the constructs by DNA sequencing, they were used to transform *Arabidopsis* plants by the floral dip procedure (Clough and Bent, 1998).

**RNA isolation and analysis**

For microarray experiments, total RNA was isolated from rosettes of 25-day-old wild-type and *P15:TCP15-EAR* plants using the RNAeasy Plant Mini Kit (Qiagen). Three biological replicates for each genotype were hybridized in Agilent Arabidopsis (V4) 4x44K arrays using two-color reciprocal labeling for two of them and one-color labeling for the third one. Labeling, hybridization, scanning and feature extraction were performed in the Ontario Cancer Institute Genomic Centre (http://www.ocigc.ca/ocigc/) according to manufacturer's protocols. Further processing of probe intensities was performed with the R statistical programming environment (R Core Team, 2013) and the limma package (Smyth, 2005) from the Bioconductor project (Gentleman *et al.*, 2004). First, probes were filtered to exclude those with log2 background intensities above 10. Intensities were then background corrected employing the "normexp" method with an offset of 16 (Silver *et al.*, 2009). Two-color arrays were normalized using the loess procedure while the two one-color arrays were quantile normalized and a MA object (Minus/Average of log2 intensities) was generated in order to combine the results. Finally, genes differentially expressed between *P15:TCP15-EAR* and wild-type plants were identified applying the empirical Bayes method (Smyth, 2005), a FDR-adjusted *p*-value of 0.05 and a threshold of 1 for the absolute value of the log2 fold change. The data are deposited in the GEO database under accession numbers GSE57742, GSE57743 and GSE57744.

For RT-qPCR analysis, RNA (three biological replicates for each line analyzed) was isolated from inflorescences using TRIzol reagent (Invitrogen). cDNA synthesis was performed from 1 µg of total RNA using MMLV reverse transcriptase (Promega) and a oligo$(dT)_{18}$ primer. Quantitative real-time PCR was performed in a StepOne (Applied Biosystems) apparatus using specific primers (Table S2) and SYBR Green detection. Gene

expression was calculated relative to actin (*ACT2* and *ACT8*; Charrier *et al.*, 2002) or *PP2AA3* (Czechowski *et al.*, 2005) using the ΔΔCt method. For transgenic plants, the results shown are from of single lines. Similar results were obtained with at least two additional lines in each case.

**Optical and scanning electron microscopy**

For optical microscopy, samples were fixed overnight at room temperature in FAA (formalin:acetyl alcohol:acetic anhydride:water, 10:50:5:35) and embedded in paraffin using standard protocols. Transverse sections (10 μm) were obtained with a rotary microtome and stained with Fast Green and Safranine. Stained samples were observed using a Mikova microscope, and photographed with a Canon A640 digital camera assembled in the microscope. For SEM, inflorescences were processed as described in Uberti-Manassero *et al.* (2012). Images were obtained in a Quanta 200 (Oregon, USA) scanning electron microscope from Centro Científico Tecnológico CONICET Rosario (Argentina).

**GUS staining**

β-Glucuronidase (GUS) activity was analysed by histochemical staining of plant tissues incubated overnight (or when satisfactory staining was obtained) at 37°C in the presence of the chromogenic substrate 5-bromo-4-chloro-3-indolyl-β-D-glucuronic acid (X-gluc) as described by Hull and Devic (1995). When GUS expression in different lines or under different conditions was compared, incubation times were the same.

**Determination of IAA levels**

IAA levels were determined using 15-day-old plants grown on MS-agar plates as described by Durgbanshi *et al.* (2005) with some modifications. Deuterated IAA ($^2H_5$-IAA; 50 ng per sample; Leibniz-Institute of Plant Biochemistry; Halle, Germany) was used as internal standard. Samples were processed as described in Andrade *et al.* (2015). IAA was quantified by quadruple tandem mass spectrometry with a negative electrospray ion source using a collision energy of 20 eV and a cone voltage of 35 V. Software MassLynx V. 4.1 was used for analysis.

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**AUTHORS' CONTRIBUTIONS**

DHG designed the project. LEL, NGUM and DHG designed the experiments and analyzed data. LEL and NGUM performed the experiments. ALA analyzed data. FC and SGA designed, performed and analyzed the determination of IAA levels. DHG wrote the manuscript. LEL and NGUM made the figures and contributed to writing. All authors corrected the manuscript.

**SUPPORTING INFORMATION**

**Figure S1.** Phenotype of *35S:TCP15* plants.

**Figure S2.** TCP15 affects silique development.

**Figure S3.** Expression of *P15:GUS* in flowers.

**Figure S4.** Expression of genes involved in auxin homeostasis and style/stigma development in *P15:TCP15-EAR* and *35S:TCP15* plants.

**Figure S5.** Phenotype of *yucca1 yucca4* flowers after cytokinin treatment.

**Table S1.** Changes in expression levels of selected genes in *P15:TCP15-EAR* plants.

**Table S2.** Oligonucleotides used in this study.

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**FIGURE LEGENDS**

**Figure 1.** Ectopic expression of *TCP15* affects carpel fusion and style/stigma development.

(a-c) Wild-type flowers.

(d,e) A *35S:TCP15* flower which lacks style and stigma and shows defects in apical carpel fusion.

(f,g) A *35S:TCP15* flower in which lack of carpel fusion extends to basal parts (arrowhead in (g)). No replum is visible between valves (f).

(h,i) *35S:TCP15* flowers with reduced stigma development, defects in carpel fusion, and exposed ovules (arrowhead in (i)).

(j-l) Flowers from *P15:TCP15-EAR* plants showing the development of outgrowths topped with stigmatic papillae from the replum.

Flowers were visualized using SEM, except for images in (c,i,l), which show photographs. Bars: 100 μm in (a,b,d-k); 1000 μm in (j).

**Figure 2.** *TCP15* is expressed in the valves and replum and affects replum development.

(a) Transverse sections of gynoecia from *P15:TCP15-EAR*, *35S:TCP15* and wild-type plants. Sections were obtained from the medial region of the gynoecium. Note the enlarged replum and septum in *P15:TCP15-EAR* plants (arrowheads).

(b) Expression of *P15:GUS* in transverse (upper panel) and longitudinal (lower panel) sections of gynoeciua. Arrowheads show *P15:GUS* expression in the valves. The arrowhead shows expression in the replum.

**Figure 3.** TCP15 modifies auxin homeostasis

(a) Expression of *DR5:GUS* in gynoecia of wild-type plants

(b) Expression of *DR5:GUS* in gynoecia of *35S:TCP15* plants. These plants show reduced GUS staining in apical and basal parts.

(c) Expression of the auxin biosynthesis genes *YUCCA1*, *YUCCA4* and *YUCCA*6 in *P15:TCP15-EAR* and *35S:TCP15* plants.

(d) Expression of the transcription factor gene *STY1* in *P15:TCP15-EAR* and *35S:TCP15* plants.

In (c) and (d), expression was analyzed by RT-qPCR in flowers of 30-day-old plants using RT-qPCR as indicated under Experimental Procedures. Error bars represent SE of three biological replicates of a single transgenic line. Similar results were obtained with two additional lines for each construct. Asterisks indicate significant differences with wild type ($P < 0.05$) according to Student's *t* tests.

**Figure 4.** TCP15 is induced by cytokinin and modulates the expression of cytokinin responsive genes.

(a) *TCP15* transcript levels in flowers of wild-type plants after a 2-h treatment with either 100 μM BAP or 25 μM NAA. Error bars represent SE of three biological replicates for each condition. The asterisk indicates a significant difference respective to control (untreated) plants ($P < 0.05$) according to a Student's *t* test.

(b) GUS staining of a plant transformed with a fusion of the *TCP15* promoter region fused to *gus* (*P15:GUS*).

(c) GUS staining of a *P15:GUS* plant treated for 2 h with 100 μM BAP.

(d) Transcript levels of genes encoding type-A response regulators ARR7 and ARR15 in wild-type (WT), *P15:TCP15-EAR* and *35S:TCP15* flowers. Error bars represent SE of three

biological replicates for each line. Asterisks indicate significant differences with wild type ($P < 0.05$) according to Student's *t* tests.

(e) GUS staining of wild-type flowers carrying a fusion of the *ARR15* promoter region to *gus* (*PARR15:GUS*)

(f) GUS staining of *PARR15:GUS* in flowers of the line shown in (e) crossed with a *35S:TCP15* plant.

**Figure 5.** Native and repressor forms of TCP15 produce different developmental responses of gynoecia to cytokinin.

(a-c) SEM analysis of flowers from wild-type plants treated with the cytokinin BAP. Cytokinin treatment causes replum enlargement and the development of outgrowths topped with stigmatic papillae.

(d,e) Flowers from *P15:TCP15-EAR* plants treated with BAP. BAP treatment causes the formation of laminar structures covered with stigma along the replum.

(f) A flower from a *35S:TCP15* plant treated with BAP showing the production of carpel- and flower-like structures from basal, medial and apical parts of the gynoecium.

(g) A flower from a *35S:TCP15* plant treated with BAP showing an ectopic flower arising from the gynophore (arrow). The arrowhead shows defective apical carpel fusion in the ectopic flower.

(h) A flower from a *35S:TCP15* plant treated with BAP showing an overproduction of undifferentiated tissues in the apical part of the gynoecium.

(i) Detail of the flower in (g) showing two unfused carpels, the development of a carpeloid structure and the presence of outgrowths in the margins (arrowhead).

(j) A close view of a carpeloid structure from (g).

(k) Detail of the apical tissues of the flower in (h) showing a sepaloid structure with stigmatic papillae in the borders.

(l) Detail of the apical tissues of the flower in (h) showing a petaloid structure with serrations and stigmatic papillae (arrowhead).

(m) An ovule-like structure developed from a petaloid of the flower in (h).

Bars: 500 µm in (a,d,f-h); 100 µm in (b,c,e); 50 µm in (i-m).

**Figure 6.** Gynoecia of mutants in *TCP14* and *TCP15* show an altered response to cytokinin.

(a) SEM analysis of a gynoecium from a wild-type plant treated with the cytokinin BAP.

(b) Gynoecium from a *tcp14* plant treated with BAP.

(c) Gynoecium from a *tcp15* plant treated with BAP. These plants show increased development of outgrowths from the replum relative to wild type.

(d) Gynoecium from a *tcp14 tcp15* plant treated with BAP. These plants show increased development of outgrowths relative to wild-type, *tcp14*, and *tcp15* plants.

(e) Enlarged replum (asterisk) in a *tcp14 tcp15* plant treated with BAP.

(f) A *tcp14 tcp15* plant treated with BAP showing the presence of irregular patches of cells in valves (arrow).

(g) Close-up of the image in (f).

(h) Replum of a wild-type gynoecium treated with BAP.

(i) Replum of a *tcp14 tcp15* gynoecium treated with BAP.

(j) Replum width in wild-type and *tcp14 tcp15* plants before and after BAP treatment. Replum width was measured from SEM images of at least six flowers from each genotype and condition. The asterisk indicates significant difference ($P < 0.05$) according to a Student's *t* test.

Bars: 500 µm in (a,e,g); 1 mm in (b-d,f); 100 µm in (h,i).

**Figure 7.** TCP15 modulates the response of auxin biosynthesis genes to cytokinin.

(a) Transcript levels of the auxin biosynthesis genes *YUCCA1* and *YUCCA4* in wild-type (WT) and *P15:TCP15-EAR* plants treated with either water (mock) or 100 μM BAP.

(b) Expression of the auxin reporter *DR5:GUS* in *P15:TCP15-EAR* plants before and after cytokinin treatment. GUS staining is observed in the ectopic structures that develop from the replum in *P15:TCP15-EAR* plants either before or after BAP treatment. The upper right panel is a close-up of the central panel showing the basal portion of the gynoecium.

(c) Transcript levels of the auxin biosynthesis genes *YUCCA1* and *YUCCA4* in wild type (WT) and *tcp14 tcp15* mutant plants treated with either water (mock) or 100 μM BAP.

Values in (a,c) are expressed relative to wild-type mock-treated plants. Error bars represent SE of three biological replicates for each line and condition. Different letters indicate significant differences ($P < 0.05$) according to ANOVA.

**Figure 8.** Changes in the activity of auxin biosynthesis genes influence the effect of cytokinin on gynoecium development.

(a) SEM analysis of a flower from a *35S:YUC1* plant.

(b,c) Flowers from a *35S:YUC1* plant treated with the cytokinin BAP. These flowers develop tubular and laminar structures from the replum.

(d) Inflorescence from a *yucca1 yucca4* mutant.

(e) Flower from a *yucca1 yucca4* plant treated with BAP. Flowers from this mutant show defects in carpel fusion in the apical part of gynoecia as a consequence of hormone treatment.

(f-h) Gynoecia from *yucca1*/+ *yucca4*/+ hemizygous mutants treated with BAP. BAP produces lack of apical fusion. The development of outgrowths is less pronounced that in

wild-type plants and is restricted to the apical portion of the gynoecium. Gynoecia are normal before BAP treatment.

Bars: 1 mm in (a-d); 500 µm in (e-h).

**Figure 9.** The effect of TCP15 on gynoecium development depends on the activity of auxin biosynthesis genes.

(a) Phenotype of *P15:TCP15-EAR* plants in an otherwise wild-type (left panel) or *yucca1 yucca4* background (right panel). The hyponastic leaf phenotype typical of *P15:TCP15-EAR* plants is preserved in the *yucca* mutant background.

(b) SEM analysis of flowers from a *P15:TCP15-EAR* plant in the *yucca1 yucca4* background. Flowers are similar to those observed in the *yucca* mutant in wild-type *TCP15* background.

(c,d) SEM analysis of gynoecia from *P15:TCP15-EAR* plants in a *yucca1/+ yucca4/+* hemizygous mutant background. Gynoecia may be similar to a wild-type (c) or have altered valve development (d), according to the severity of the phenotypic changes caused by *TCP15-EAR* expression, but the development of outgrowths is inhibited in both types of plants.

(e) SEM analysis of a gynoeciium from a *P15:TCP15-EAR* plant in a *yucca1/+ yucca4/+* hemizygous mutant background after BAP treatment. The development of outgrowths is reduced in comparison with *P15:TCP15-EAR* plants in a wild-type background.

(f) SEM analysis of a flower from a *35S:TCP15 35S:YUC1* plant treated with the cytokinin BAP. These plants show normal development of apical tissues. Laminar structures typical of *35S:YUC1* plants arise from the replum (arrow). The development of a laminar carpeloid structure from the gynophore is also observed (arrowhead).

(g) A flower as in (d) showing the development of laminar structures typical of *35S:YUC1* plants (arrow) and the production of an extra flower from the gynophore (arrowhead).

Bars: 1 mm in (b,c,e); 500 µm in (d,f,g).

**Figure 10.** TCP15 and related proteins participate in a regulatory loop that links auxin and cytokinin responses during gynoecium development in Arabidopsis.

Cytokinin (CK) treatment causes increased replum development (i; Marsch-Martinez *et al.*, 2012; this work) and induces and activates TCP15 (ii; Steiner *et al.*, 2012; this work). TCP15 affects auxin homeostasis and inhibits the expression of auxin biosynthesis genes (iii; Uberti-Manassero *et al.*, 2012; this work). A role of auxin (AUX) in medial tissue development has been suggested, and plants with altered *YUCCA* gene expression respond differently to cytokinin treatment (iv; Nemhauser *et al.*, 2000; Girin *et al.*, 2009; Sorefan *et al.*, 2009; this work). In the absence of TCP15 and TCP14, cytokinin treatment leads to increased expression of auxin biosynthesis genes (v; this work). Accordingly, cytokinin would lead to different developmental responses in gynoecium tissues according to auxin levels or homeostasis and TCP15 action.