Control of embryonic meristem initiation in *Arabidopsis* by PHD-finger protein complexes

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**SUMMARY**

Plant growth is directed by the activity of stem cells within meristems. The first meristems are established during early embryogenesis, and this process involves the specification of both stem cells and their organizer cells. One of the earliest events in root meristem initiation is marked by re-specification of the uppermost suspensor cell as hypophysis, the precursor of the organizer. The transcription factor MONOPTEROS (MP) is a key regulator of hypophysis specification, and does so in part by promoting the transport of the plant hormone auxin and by activating the expression of TARGET OF MP (TMO) transcription factors, both of which are required for hypophysis specification. The mechanisms leading to the activation of these genes by MP in a chromatin context are not understood. Here, we show that the PHD-finger proteins OBERON (OBE) and TITANIA (TTA) are essential for MP-dependent embryonic root meristem initiation. TTA1 and TTA2 are functionally redundant and function in the same pathway as OBE1 and OBE2. These PHD-finger proteins interact with each other, and genetic analysis shows that OBE-TTA heterotypic protein complexes promote embryonic root meristem initiation. Furthermore, while MP expression is unaffected by mutations in *OBE/TTA* genes, expression of MP targets *TMO5* and *TMO7* is locally lost in *obe1* *obe2* embryos. PHD-finger proteins have been shown to act in initiation of transcription by interacting with nucleosomes. Indeed, we found that OBE1 binds to chromatin at the *TMO7* locus, suggesting a role in its MP-dependent activation. Our data indicate that PHD-finger protein complexes are crucial for the activation of MP-dependent gene expression during embryonic root meristem initiation, and provide a starting point for studying the mechanisms of developmental gene activation within a chromatin context in plants.

**KEY WORDS:** *Arabidopsis*, PHD finger, Embryogenesis

**INTRODUCTION**

Apical meristems, located at the growing tips, are indispensable for plant development because these produce all plant organs post-embryonically (Weigel and Jürgens, 2002). The meristems and the stem cells contained within these are formed during embryogenesis. The first manifestation of embryonic root meristem initiation is marked by the specification of an initially extra-embryonic suspensor cell as hypophysis. The hypophysis divides asymmetrically and its small descendant cell will become the quiescent center (QC), which maintains stem cell identity in adjoining cells of the root meristem (reviewed by Möller and Weijers, 2009). Root meristem initiation has been studied mostly using genetic approaches. Few mutations that specifically affect embryonic root initiation have been identified (Mayer et al., 1991), and most of those that have been described converge on the activity of the auxin-dependent transcription factor MONOPTEROS (MP)/AUXIN RESPONSE FACTOR 5 (Hardtke and Berleth, 1998; Weijers et al., 2006) (reviewed by Möller and Weijers, 2009). MP is inhibited by the interacting BODENLOS (BDL)/IAA12 protein. The plant hormone auxin promotes degradation of BDL, thereby releasing MP from inhibition (Hamann et al., 2002). Knowledge about the network operating downstream of MP in root initiation, and the mechanisms of gene regulation by MP beyond inhibition by BDL is fragmented. Recently, a first set of MP targets was identified. Among these, TARGET OF MP 5 (*TMO5*) and *TMO7* genes are directly activated by binding of MP to their promoters. In turn, *TMO7* is required for MP-dependent embryonic root meristem initiation (Schlereth et al., 2010). MP also promotes the transport of auxin through controlling PIN1 activity, resulting in auxin accumulation in the future hypophysis that is a fundamental event for MP-dependent embryonic root meristem initiation (Friml et al., 2003; Weijers et al., 2006). In addition to MP and its direct target *TMO7*, several other factors have been shown to contribute to embryonic root formation. PLETHORA (PLT) proteins, which belong to the AP2-type transcription factor family, are essential for the specification and maintenance of the stem cells (Aida et al., 2004; Galinha et al., 2007), while GRAS family transcription factors SCARECROW (SCR) and SHORT-ROOT (SHR) are important for controlling the radial tissue organization of the root (Di Laurenzio et al., 1996; Helariutta et al., 2000; Sabatini et al., 2003). Although all these transcription factors have been shown to be involved in embryonic root meristem formation, their activity appears to be required after initial MP-dependent initiation. Key unanswered questions are what the connections between these components are, and what mechanisms ensure strict spatial control of these genes.

*OBERON1 (OBE1)* and OBE2 genes encode plant homeodomain (PHD)-finger proteins and these genes act redundantly in MP-dependent embryonic root initiation (Saiga et al., 2008; Thomas et al., 2009). The PHD-finger domain is found in a wide variety of proteins involved in the regulation of chromatin structure (Taverna et al., 2007). PHD-finger domain is constituted of a conserved Cys4-His-Cys3 zinc-finger domain (Aasland et al., 1995). Recent studies demonstrated that the PHD-
finger domain specifically binds to histone H3 trimethylated at lysine 4 (Li et al., 2006; Peña et al., 2006; Shi et al., 2006; Wysocka et al., 2006; Lee et al., 2009), which is associated with nucleosomes near the promoters and 5’ ends of highly transcribed genes (Zhang et al., 2009), and recruit transcription factors and nucleosome-associated protein complexes to chromatin (Saksouk et al., 2009). Interestingly, although PLT1, PLT2, SCR and WOX5 are not expressed in obe1 obe2 double-mutant embryos, MP is normally expressed. As expression of PLT1, PLT2 and WOX5 depends on MP (Aida et al., 2004; Sarker et al., 2007), one possibility is that OBE1 and OBE2 act to control embryonic root meristem formation downstream or at the level of MP (Saiga et al., 2008). However, the function of OBE proteins in the MP pathway is not known.

Here, we demonstrate the role of PHD-finger proteins involved in MP-dependent embryonic root initiation in Arabidopsis. TITANIA1 (TTA1) and TTA2 genes, which are closest homologs of OBE1 and OBE2, are functionally redundant and required for MP-dependent embryonic root initiation. Our data show that OBE1 locally mediates the activation of TMOS and TM07 genes. Construction of triple and quadruple mutants among obe1, obe2, tta1 and tta2 showed that OBE1/2 and TTA1/2 also act redundantly in embryogenesis. Our findings suggest that activation of transcription factor genes during root initiation requires the activity of a PHD-finger protein complex.

RESULTS

TTA1 and TTA2 are essential for normal pattern formation

We have previously demonstrated that the PHD-finger proteins OBE1 and OBE2 are indispensable for the establishment and maintenance of the both shoot and root apical meristem (RAM and SAM, respectively) (Saiga et al., 2008). In Arabidopsis, there are two close homologs of OBE1 and OBE2, and we named these TITANIA1 (TTA1) and TTA2 (Fig. 1A). TTA1 (At1g14740) and TTA2 (At3g63500) proteins share 55% amino acid similarity, suggesting that TTA1 and TTA2 function redundantly, as is the case of OBE1 and OBE2. To test this possibility, we analyzed loss-of-function mutants of TTA1 (ttal-1) and TTA2 (ttal-2 and ttal-2) (supplementary material Fig. S1A). As none of single mutants exhibited obvious phenotypes (data not shown), we generated double mutant combinations of these mutants. All ttal ttal double mutants showed seedling lethality (supplementary material Fig. S1B,C) and these phenotypes were completely rescued by introducing TTA1p::TTA1-GFP or TTA2p::TTA2-GFP (supplementary material Fig. S1D; data not shown). These observations indicate that TTA1 and TTA2 indeed function redundantly. We used ttal-1 as the ttal mutant for all further analyses.

ttal ttal double mutants exhibited a rootless phenotype and this defect is probably derived from disruption of normal pattern formation during embryogenesis. To determine how embryonic pattern formation is perturbed in ttal ttal, we examined embryos from self-fertilized plants heterozygous for ttal and homozygous for ttal as double homozygous plants died before flowering. It is expected that ~25% of embryos from these plants might segregate as ttal ttal double homozygous.

We found additional and abnormal cell divisions at the embryo proper from the two-cell to 16-cell stage (Fig. 1B,F; data not shown). Although it was observed in only a fraction of embryos from ttal-1/ ttal-2 plants (Table 1), both TTA1p::TTA1-GFP and TTA2p::TTA2-GFP completely rescued those defects (data not shown), indicating that deprivation of both TTA1 and TTA2 is responsible for those phenotypes. At the globular stage, during which the hypophysis divides into a smaller apical cell and larger basal cell in the wild-type embryo (size of apical cell, 5.7±0.5 μm; size of basal cell, 11.1±0.3 μm; n=20), the hypohysis of ttal ttal embryos divided abnormally (Fig. 1C,G), resulting in production of two equally size descendants (size of apical cell, 8.1±0.6 μm; size of basal cell, 9.2±0.5 μm; n=20) (Fig. 1D,H). Furthermore, cell
division of endodermis/cortex cell files in 
tta1 tta2 is missing (Fig.
1E,I). These observations suggest that the rootless phenotype in
the tta1 tta2 double mutant results from an early defect in embryonic
root initiation. After germination, tta1 tta2 seedlings have a
variable number of cotyledons (Fig. 1J,K; Table 2) in addition to
the rootless phenotype and eventually die after forming the first
pair of leaves (Fig. 1L,M).

**TTA1 and TTA2 are required for root meristem patterning**

To address whether only cell division is affected in tta1 tta2 embryos, or whether cell identities are incorrectly specified, we
examined the expression of marker genes by in situ hybridization
or fluorescence microscopy-based expression analysis. In this
analysis, we used embryos obtained from self-fertilized plants
heterozygous for tta1 and homozygous for tta2, in which it is
expected ~25% of embryos might segregate as tta1 tta2 double
homozygous.

As the hypophysis division defect in tta1 tta2 embryos strongly
resembles the mp mutant, we first addressed whether MP
expression is lost in tta1 tta2 embryos. Ninety-six percent (23 out
of 24) of early globular stage embryos showed wild-type MP
expression (data not shown). At the heart stage, MP was still
expressed in the tta1 tta2 embryos (Fig. 2A,E), suggesting that the
phenotype is not due to a loss of MP expression. We next
investigated the expression of the PLT1 and SCR genes. PLT1 is
required for the QC specification and is expressed in the basal
region of embryo proper at the globular stage in wild type (Fig. 2B)
(Aida et al., 2004). However, no PLT1 expression was detectable
in 25% (11/44) of embryos (Fig. 2F). SCR is also required for the
QC specification, in which it acts in parallel with PLT genes
(Sabatini et al., 2003; Aida et al., 2004). SCR is initially expressed
in the hypophysis at the early globular stage and is subsequently
activated in the ground tissue (Fig. 2C) (Wysocka-Diller et al.,
2000). By contrast, of the globular stage embryos, tta1 tta2
embryos (14/57) failed to express SCR (Fig. 2G). These data
dicate that specification of the QC is defective in tta1 tta2 embryos.
Interestingly, SCR expression was also lost from ground
tissue cells (Fig. 2G), which is consistent with the failure of these
cells to divide in the double mutant (Fig. 1I). Consistent with a loss
of QC identity, WOX5 expression, which initiates in the hypophysis
at the globular stage and subsequently becomes restricted in the
lens-shaped cell and its derivatives in the wild-type embryo (Fig.
2D) (Haecker et al., 2004), was completely lost in tta1 tta2
embryos (8/30) (Fig. 2H). In summary, MP was still expressed in
tta1 tta2 embryos, but expression of PLT1, SCR and WOX5 was
lost, suggesting that cell identity specification in this mutant is
compromised downstream of MP activity.

**TTA1 and TTA2 are expressed ubiquitously during embryogenesis**

As TTA1 and TTA2 are redundantly required for specification of
the hypophysis and establishment of the embryonic root, we
predicted that TTA1 and TTA2 proteins are expressed in the basal
region of embryo proper and/or upper-most suspensor cells at the
early globular stage, when the hypophysis is specified (Weijers et
To investigate the expression pattern of TTA1 and TTA2 proteins, we generated TTA1p::TTA1-GFP and TTA2p::TTA2-GFP transgenic lines, and analyzed the expression pattern of these throughout embryonic development. Both constructs complemented the double mutant phenotype (supplementary material Fig. S1D; data not shown), indicating that the fusions encode functional proteins. GFP fluorescence was first detected in the two-cell stage embryos (Fig. 3A). At the early globular stage, TTA1-GFP was found both in the basal region of embryo proper and suspensor cells (Fig. 3B), which is consistent with the finding that TTA1 has a role for hypophysis specification. During embryonic development, TTA1 was expressed not only in the basal region but also in the apical region of the embryo proper (Fig. 3C,D). TTA2 displayed the same expression pattern at all stages examined (Fig. 3E-H). Interestingly, despite the ubiquitous expression of both genes, the phenotype resulting from the loss of both genes is remarkably specific to the hypophysis.

TTA and OBE function in the same pathway through forming a heterotypic protein complex

Given the finding that tta1 tta2 exhibited similar defects as observed in obel obe2 embryos (compare with Saiga et al., 2008), we generated multiple mutant combinations among these mutants. We found that obel tta1, obe1 tta2, obe2 tta1 and obe2 tta2 double mutants exhibited no obvious phenotypes. This result suggests that the OBE1/2 and TTA1/2 proteins are not simply redundant, but rather that one protein from each pair is required for normal development. To determine the consequences of progressively eliminating the entire OBE1/2 TTA1/2 clade, we next analyzed embryos of obel obe2 tta2 triple mutants from an obel/+ obe2 tta2 mother plant. Among the progeny of such plants, 20% of embryos exhibited embryonic lethality (Table 3). obe1 obe2 tta2 did not show novel phenotypes in the basal region where the formation of the embryonic root meristem is already disrupted in obel obe2 and tta1 tta2 embryos. However, development of the apical region where cotyledon primordia and shoot apical meristem are produced was disturbed (Fig. 4A-C,E-G). During transition from triangular to heart stage in wild-type siblings in the same silique, cotyledon primordia had correctly emerged (Fig. 4A,B); however, emergence of cotyledon primordia was not observed in obel obe2 tta2 embryos (Fig. 4E,F). In addition, the apical region of obel obe2 tta2 embryos was abnormally expanded compared with wild-type siblings (Fig. 4B,F). obe1 obe2 tta2 triple mutant embryos arrested at the triangular stage (Fig. 4C,G). We further investigated other triple mutant combinations and found that all of them showed same phenotypes (data not shown). Finally, we investigated the phenotypes of obel obe2 tta1 tta2 quadruple mutants. We found that ~5% of embryos from obel+/+ obe2 tta1+/+ tta2 mother plants were swollen when wild-type siblings were at the bent-cotyledon stage (Fig. 4D,H; Table 3). These data indicate that although the OBE1/2 and TTA1/2 pairs are not redundant in root formation, all four proteins function redundantly in development of the apical pole, as well as in progression beyond the triangular stage of embryogenesis.

The genetic interactions seen among OBE1/2 and TTA1/2 proteins are consistent with joint requirement of multiple proteins for biological function, for example, in a protein complex. To determine whether protein complexes can be formed among these proteins, we initially tested all possible pairwise combinations between OBE and TTA proteins, including their homodimers, using a yeast two-hybrid assay. In this assay, all tested interactions were positive (Fig. 5A), suggesting extensive interaction potential among all proteins.
To determine whether complexes involving multiple OBE/TTA proteins are found in vivo, we used a translational fusion construct for OBE1-GFP that was previously shown to be functional (Saiga et al., 2008). We isolated the OBE1-GFP protein complex using immunoprecipitation on silique tissue. Next, associated proteins were identified by tandem mass spectrometry. Strikingly, in addition to OBE1, peptides uniquely representing OBE2, TTA1 and TTA2 were identified in pull-down experiments with OBE1-GFP siliques, but not with wild-type siliques (Fig. 5B; Table 4). Given the finding that TTA and OBE proteins act downstream or at the level of MP, we analyzed the mass spectrometry results for MP peptides, but did not find any. Hence, there is no evidence for a direct association between OBE1 and MP. These results demonstrate that OBE1 is found in complex with other OBE/TTA proteins, although this experiment does not resolve the size or topology of such complexes.

OBE1 is required for the expression of direct MP target genes

We have shown previously that obe1 obe2 double mutants are defective in embryonic root initiation, resulting in rootless phenotype similar to mp mutants (Saiga et al., 2008). In obe1 obe2 embryos, the expression of PLT1 is lost; however, MP is still expressed, suggesting that OBE1 and OBE2 act downstream or at the level of MP.

Recently, TMOS and TM07 genes, both of which encode bHLH type transcription factors, have been identified as direct targets of MP that mediate MP-dependent embryonic root initiation (Schlereth et al., 2010). To determine whether OBE1 is involved in the regulation of these direct MP target genes, we examined TMOS and TM07 expression in obe1 obe2 embryos. TMOS is expressed in cells adjacent to the hypophysis and in cotyledon primordia in wild type (Fig. 6A). Although the expression in the lower domain of the embryo is abolished in obe1 obe2 embryos, the apical expression is maintained (Fig. 6C). The expression of TM07, which is expressed in the hypophysis-adjacent cells in wild type (Fig. 6B), was completely abolished in obe1 obe2 embryos (Fig. 6D). These findings indicate that OBE1 and OBE2 are required for the expression of TMOS and TM07 genes in cells adjacent to the hypophysis. In addition, the observation that only basal expression of TMOS was abolished in obe1 obe2 embryos suggests different requirements for OBE1/2 gene activity in root and cotyledon patterning, as was also suggested by the genetic analysis.

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**Table 3. Combinations of obe1, obe2, tta1 and tta2 mutants**

<table>
<thead>
<tr>
<th>Parental genotype</th>
<th>Normal*</th>
<th>Triangular arrest</th>
<th>Swollen embryo</th>
<th>n</th>
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<tr>
<td>Wild type</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>190</td>
</tr>
<tr>
<td>obe2 tta1/ tta2</td>
<td>77</td>
<td>23</td>
<td>0</td>
<td>337</td>
</tr>
<tr>
<td>obe1+ obe2 tta1</td>
<td>81</td>
<td>19</td>
<td>0</td>
<td>202</td>
</tr>
<tr>
<td>obe1+ obe2 tta1/ tta2</td>
<td>60</td>
<td>35</td>
<td>5</td>
<td>446</td>
</tr>
</tbody>
</table>

*Frequency (%) of embryos indistinguishable from wild type. n, number of embryos analyzed.

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**Fig. 4. Phenotypes of triple and quadruple mutants.**

(A–H) Embryonic phenotypes of wild-type siblings (A–D), obe1 obe2 tta2 (E–G) and obe1 obe2 tta1 tta2 (H) at the late globular (A,E), triangular (B,F), heart (C,G) and bent-cotyledon (D,H) stages. Arrows indicate the positions where cotyledon primordia have emerged. Arrowheads indicate the positions where shoot apical meristem are formed. Scale bars: 30 μm in A–C,E–G; 100 μm in D,H.

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**Fig. 5. Interactions between OBE1/2 and TTA1/2 proteins.**

(A) Yeast strain YRG-2 harboring the indicated plasmids were grown on medium lacking tryptophan, leucine and histidine. (B) Sequence of OBE1 protein with all highlighted peptides (in grey) that have been identified in the mass spectrometry analysis of at least one of three independent immunoprecipitation experiments. Note that peptides are found from across the entire protein, indicating that intact proteins were precipitated (399/566 amino acids, 70.5%).
As MP is still expressed, but not TMO7 (the direct target of MP) in obe1 obe2 embryos, we hypothesized that OBE1 and OBE2 mediate the TMO7 expression through modification of, or binding to, chromatin at the TMO7 locus. To confirm the association of OBE1 with TMO7 promoter region, we performed chromatin immunoprecipitation (ChIP) analysis with the OBE1p::OBE1-GFP transgenic line that could rescue the defects of obe1 obe2. Three DNA fragments in the TMO7 promoter region were enriched using a GFP antibody (Fig. 6E,F; supplementary material Fig. S2), demonstrating in vivo binding. Interestingly, the binding profile of OBE1 along the tiles chosen for the TMO7 locus closely resembled that of MP binding, as previously demonstrated (Schlereth et al., 2010), suggesting that a functional interaction may exist.

It has been demonstrated that while the TMO7 transcript is expressed in the cells adjacent to the hypophysis, the TMO7 protein moves to the hypophysis where it acts to mediate root formation (Schlereth et al., 2010). If OBE1 mediates root formation in part by controlling TMO7, one would predict a requirement for OBE1 in the cells adjacent to the hypophysis but not in the hypophysis itself. As OBE1 is ubiquitously expressed at this stage (Saiga et al., 2008), it cannot be deduced where its activity is required. To determine the domain of OBE1 activity in root formation, we misexpressed OBE1 in obe1 obe2 mutants from two different promoters, and observed embryonic root initiation of those plants. OBE1 driven by MP promoter, which is expressed in the cells adjacent to the hypophysis but not in the hypophysis itself (Schlereth et al., 2010), could rescue the embryonic root initiation defects in obe1 obe2 (Fig. 6G). By contrast, OBE1 expression driven by the suspensor-specific ARF13 promoter (Schlereth et al., 2010) in ARF13p::OBE1 lines did not rescue the defects in obe1 obe2 roots (data not shown). These data indicate that OBE1 in the cells to the adjacent to the hypophysis is crucial for the embryonic root initiation, and OBE1 is important for TMO7 expression but not for its protein function.

**DISCUSSION**

Our results indicate that TTA1 and TTA2 are redundantly required for embryonic root initiation in Arabidopsis. The observations that cell divisions of the hypophysis of tta1 tta2 are defective, and that MP is expressed but PLT1, SCR and WOX5 are absent in tta1 tta2 embryos suggest that the rootless phenotype observed in tta1 tta2 is mainly derived from disruption of the hypophysis specification. TTA1 and TTA2 seem to function in the same pathway in which OBE1 and OBE2 act because: (1) phenotypes of tta1 tta2 double mutants are similar to those of obe1 obe2; (2) the expression patterns of cell identity marker genes are identical to those of obe1 obe2; (3) expression patterns of all four proteins completely overlap; and (4) OBE1/2 and TTA1/2 proteins could interact with each other in vivo.

Because MP is present in the adjacent cells to the future hypophysis but not in the hypophysis itself, it follows that MP promotes the hypophysis specification in a non-cell-autonomous manner (Hardtke and Berleth, 1998). TMO7 expression is activated by MP in the adjacent cells to the hypophysis and TMO7 protein moves to the hypophysis. Our findings indicate that OBE1 mediates the MP-dependent TMO7 expression because: (1) the expression of TMO7 but not MP is lost in obe1 obe2 embryos; (2) OBE1 associates with the TMO7 promoter region; and (3) OBE1 function in the adjacent cells to the hypophysis but not in the hypophysis is required for embryonic root initiation (Fig. 7). OBE1, OBE2, TTA1 and TTA2 expression seem not to be regulated by MP, and protein complex identification with either OBE1 or MP failed to detect

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**Table 4. Identification of OBE1 and interacting proteins by immunoprecipitation**

<table>
<thead>
<tr>
<th>AGI</th>
<th>Protein name</th>
<th>Experiment 1</th>
<th>Experiment 2</th>
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<tbody>
<tr>
<td></td>
<td>n</td>
<td>%</td>
<td>Sf</td>
</tr>
<tr>
<td>At3g07780</td>
<td>OBE1</td>
<td>40 (37)</td>
<td>62</td>
</tr>
<tr>
<td>At5g48160</td>
<td>OBE2</td>
<td>8 (5)</td>
<td>17</td>
</tr>
<tr>
<td>At1g14740</td>
<td>TTA1</td>
<td>17 (17)</td>
<td>25</td>
</tr>
<tr>
<td>At3g63500</td>
<td>TTA2</td>
<td>22 (22)</td>
<td>23</td>
</tr>
</tbody>
</table>

Identification of OBE1 and interacting proteins from immunoprecipitations with 1 g of siliques expressing OBE1-GFP under the control of its endogenous promoter. Two independent pull-down experiments have been performed. Control experiments with wild-type siliques were performed at the same time, and, subsequently, all samples were subjected to mass spectrometry analysis. The peptides found with pull-down experiments with wild-type and OBE1-GFP siliques were compared, and all shared peptides were excluded for further analysis.

n, number of different peptides (number of unique peptides); %, percentage coverage of protein; Sf, total score factor calculated using Bioworks v3.3.1.

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**Fig. 6. OBE1 mediates the expression of MP target genes.**

(A,C) TMO5p: 3×nGFP expression in wild-type (A) and obe1 obe2 (C) embryos. (B,D) TMO7p: 3×nGFP expression in wild-type (B) and obe1 obe2 (D) embryos. (E) Schematic representation of the TMO7 promoter region. The thin line and black boxes represent non-coding regions and exons, respectively. Bars with numbers illustrate the DNA fragments amplified in F. (F) PCR-amplified TMO7 promoter fragments from ChIP of OBE1p::OBE1-GFP embryos with (An) or without (No) anti-GFP antibody. In, chromatin input. (G) Phenotypes of MPp::OBE1 in obe1 obe2 plants at 6 days after germination. From left to right: wild type, obe1 obe2 and obe1 obe2 harboring MPp::OBE1. Scale bars: 30 μm in A-D; 10 mm in G.
Acetylation allows transcription factors to access target genes. Decondensation of nucleosome structure mediated by histone remodeling complex such as histone acetyltransferase (HAT), and possibility is that OBE proteins are contained in a chromatin structure, suggesting that accumulation of both auxin and TMO7 is required for the hypophysis specification. The precise molecular mechanisms for this regional MP activity remain to be determined, but the OBE proteins should allow dissecting these.

All triple mutant combinations among obe1, obe2, tta1 and tta2 exhibited no additional phenotypes in the formation of embryonic root meristem that are already disrupted in obe1 obe2 and tta1 tta2 double mutants, whereas development of apical region in triple mutants displayed more severe phenotypes than those of double mutants, indicating that OBE1/2 and TTA1/2 function in development of embryonic shoot meristem and cotyledons synergistically. Previously, we have demonstrated that the embryonic shoot meristem of obe1 obe2 might be formed initially but is not maintained because the expression of shoot meristem marker genes WUSCHEL and CLAVATA3 in obe1 obe2 embryos is initiated but is not maintained. By contrast, the embryonic root meristem of obe1 obe2 was not formed, as judged by the expression patterns of root meristem marker genes (Saiga et al., 2008). These suggest that a more complex mechanism operates in the embryonic shoot meristem and cotyledon development, as was also suggested by the differential effect of obe1 obe2 mutations on TMO7 expression in the two embryo poles.

The recent identification of TMO genes as MP targets provides entry points to connect the upstream regulator MP with its several downstream pathways. A key question is how region-specific MP activity is controlled. The analysis of TMO5 and TMO7 expression in obe1 obe2 mutants provides insight into this problem. Although TMO7 is eliminated entirely, TMO5 expression is lost only in the basal embryo domain. This suggests that the requirements for gene activation by MP in the basal and apical embryo domains differ. The precise molecular mechanisms for this regional MP activity remain to be determined, but the OBE proteins should allow dissecting these.

Auxin is another signal involved in this signaling but its accumulation alone is not sufficient to promote the hypophysis specification (Weijers et al., 2006). Whereas auxin response is activated in extra-embryonic cells below the future hypophysis, TMO7 protein exists only in uppermost extra-embryonic cell, suggesting that accumulation of both auxin and TMO7 is required for the hypophysis specification (Schlereth et al., 2010). TMO7 expression is absent in obe1 obe2 embryos, whereas the establishment of auxin response maxima in obe1 obe2 embryos is largely similar to the wild-type pattern (Thomas et al., 2009) (S.S., M.A., D.W. and Y.K., unpublished), suggesting that OBE1 mainly controls the expression of the TMO7 rather than establishment of the auxin maxima in the hypophysis specification.

The observation that all triple mutant combinations exhibited more severe phenotypes than those of double mutants is curious because both TTA1 and TTA2 and OBE1 and OBE2 are functionally redundant. One possible explanation is that there are differences in functionality among dimers containing OBE1/2 or TTA1/2. During embryogenesis, hetero-dimer formation might be important. For example, the obe1 obe2 tta1 triple mutant should only have TTA2 homo-dimer, and this results in embryo lethality. However, the obe1 obe2 double mutant, in which TTA1-TTA2 hetero-dimer can exist, can form cotyledons and germinate. However, the obe1 obe2 and tta1 tta2 double mutants have no OBE-TTA hetero-dimers and functional embryonic apical meristems were not established. Taken together, our results indicate that OBE-TTA dimer formation might be most important for Arabidopsis embryogenesis. More detailed analysis should elucidate how the OBE-TTA protein complex acts in the apical region during embryonic development. Finally, this work opens up avenues for studying the regulation of developmentally important genes through transcription factors and chromatin proteins.

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Competing interests statement
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Supplementary material
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References


