Minireview

The *topless* plant developmental phenotype explained!

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Published: 30 April 2008

*Genome **Biology*** 2008, **9:**219 (doi:10.1186/gb-2008-9-4-219)

The electronic version of this article is the complete one and can be found online at http://genomebiology.com/2008/9/4/219

Abstract

The molecular-genetic cues that regulate plant embryo pattern formation are the subject of intense scrutiny at present. Recent work in *Arabidopsis* implicates the TOPLESS protein in auxin-dependent transcriptional repression, highlighting once again the crucial role of auxin signaling during embryogenesis.

Biologists have long been fascinated by the question of how body patterns are specified during embryogenesis, starting from an undifferentiated zygote. In animals, embryogenesis typically lays down the adult body plan, whereas in higher plants, embryogenesis produces a minimal plant - the seedling. Consequently, the vast majority of adult organs in plants are only initiated during post-embryonic growth. Nevertheless, embryogenesis is of pivotal importance for adult plant development, because post-embryonic growth is driven by pools of stem cells established during embryogenesis. These are laid down in the primary seedling meristems and yield the above- and below-ground structures, such as leaves and roots, of the adult.

In the model plant *Arabidopsis thaliana*, embryogenesis involves highly patterned and predictable cell divisions. The first zygotic division is asymmetric, generating a smaller, apical cell from which most of the embryo proper will be derived, and a second, basal cell that gives rise to the suspensor and part of the root meristem and cap [1]. A subsequent series of highly stereotypic divisions eventually leads to formation of embryonic tissues along a polarized apical-basal axis and a radial axis. A large body of work has been performed to identify key players in this patterning process.

In this context, an interesting temperature-sensitive mutant, *topless* (*tpl*), forms apical roots instead of shoots in the embryo at a restrictive temperature [2]. Consistent with this phenotype, expression of apical markers is progressively lost in *tpl* embryogenesis, whereas expression of root fate markers extends into the apical region [2]. *tpl* mutants undergo a homeotic transformation from apical to basal cell fate in the presumptive shoot. Work recently published in *Science* by Szemenyei *et al.* [3] now shows that TOPLESS is involved in auxin-dependent transcriptional repression during embryogenesis in *Arabidopsis*.

The role of mediators of auxin activity in plant embryogenesis

A number of factors that relay the local activity of the plant hormone auxin in plant development have been identified [4-9]. They include factors that actively regulate auxin distribution, such as PIN-FORMED (PIN) proteins, as well as transcriptional regulators that convert cellular auxin concentration into gene-expression responses. PIN-dependent auxin transport is essential for embryonic axis formation [6], as is cellular auxin signaling, which is intertwined with auxin transport through feedback loops [10-12]. Two key mutants in embryonic auxin signaling, *bodenlos* (*bdl*) and *monopteros* (*mp*), fail to form an embryonic root meristem and therefore yield rootless seedlings [13,14]. *BDL* encodes a transcriptional corepressor of the AUX/IAA family that inhibits the activation potential of auxin-response factor (ARF) transcription factors, such as the MP protein, by direct protein-protein interaction [7,8,15]. Several lines of investigation have demonstrated that AUX/IAA proteins

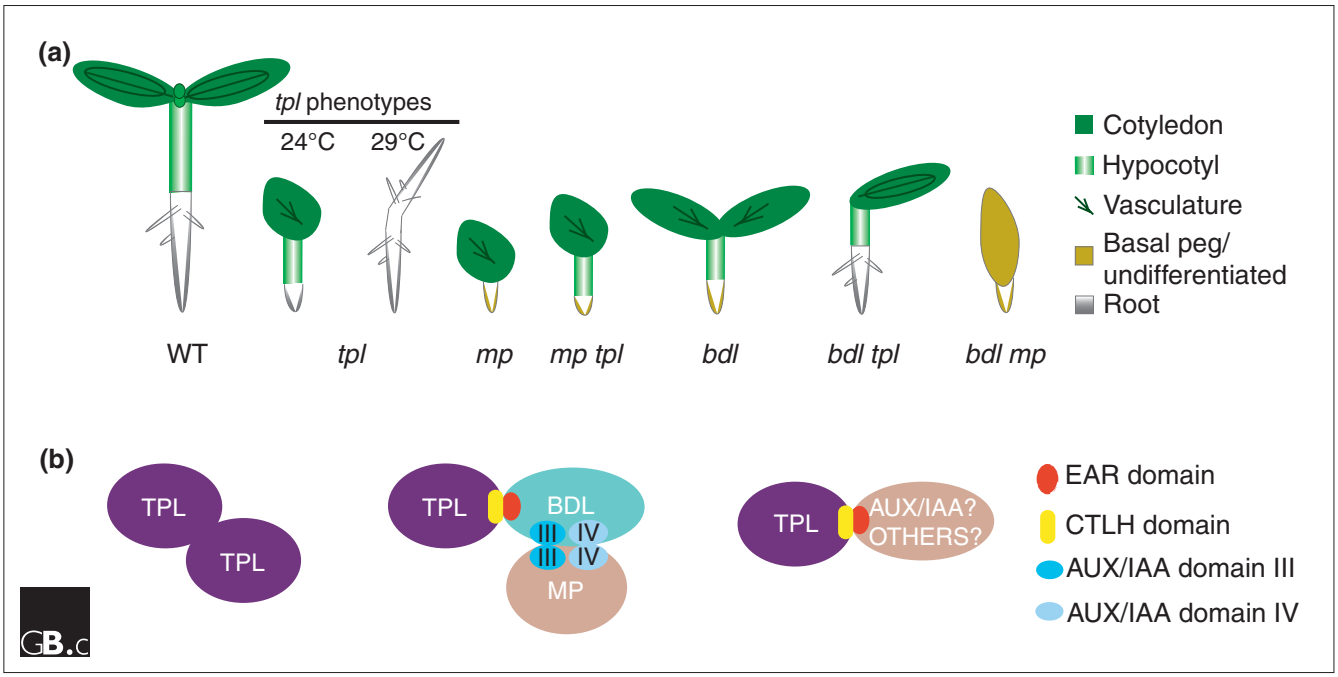

Figure 1
Mutations that affect auxin-mediated apical-basal patterning in *Arabidopsis* and the role of TOPLESS. **(a)** Schematic diagram of wild-type (WT), *topless* (*tpl*), *monopteros* (*mp*), *bodenlos* (*bdl*), *mp tpl*, *bdl tpl*, and *bdl mp* seedling phenotypes. The temperature-sensitive *tpl* mutant displays apical patterning defects at permissive temperatures (24°C), whereas restrictive temperatures (29°C) result in complete homeotic transformation of the seedling shoot to root. In the double mutants, *tpl* can suppress patterning defects in both *mp* and *bdl*. **(b)** Proposed function of the TOPLESS (TPL) corepressor protein in the nucleus. TPL can homodimerize, act in a complex with IAA12/BDL and MP, and hypothetically interact with other EAR-domain-containing proteins. The CTLH domain of TPL and the EAR domain of BDL mediate the interaction between TPL and BDL. Domains III and IV of BDL are found in all AUX/IAA proteins and mediate interaction with similar domains in ARFs, such as MP.

are degraded upon auxin-mediated interaction with auxin receptors, resulting in the release of ARFs to activate downstream targets of auxin signaling [16,17]. The dominant *bdl* mutant carries a mutation that desensitizes BDL protein to auxin-induced degradation. Similarly to the loss of MP activity in recessive *mp* mutants, this results in severely impaired auxin-dependent embryonic gene expression.

The *tpl* mutation is a rare temperature-sensitive mutation that changes apical cell fate. In the first examinations of a possible role for *TPL* in the auxin pathway by Long *et al.* [2], double mutants were generated between *tpl* and *mp*. Apically, these largely resembled *tpl* single mutants, whereas basally they resembled *mp*, although the reduced hypocotyl and vascular differentiation of *mp* was partially suppressed by *tpl* (Figure 1a). When the synthetic *DR5* reporter construct, which monitors ARF activity and thereby auxin concentration, was introduced into *tpl* embryos, it rarely displayed strong ectopic apical expression [2]. Altogether, these findings led Long *et al.* to suggest that disturbance in the auxin pathway could not be responsible for the *tpl* phenotype. However, further experiments with the TPL protein by the same laboratory [3] reveal that this assumption was premature.

TOPLESS represses auxin-induced gene expression

Molecular cloning of *TPL* indicated that the TPL protein resembles known transcriptional corepressors [18]. Consistent with this idea, TPL is localized to the nucleus and mutations in a histone acetyltransferase, a known transcriptional coactivator, suppress *tpl* phenotypes. The *tpl* mutation acts as a dominant negative, probably interfering with the activity of other TPL-related proteins [18]. The quintuple knockout of the whole *TPL*-related gene family confirmed this notion, as it phenocopies the original *tpl* semi-dominant allele [18]. *TPL* is initially expressed throughout the embryo and then restricted to the incipient vasculature, resembling the expression patterns of *MP* and *BDL* [18].

Szemenyei *et al.* [3] have now found that TPL interacts directly with BDL and indirectly, by BDL bridging, with MP *in planta*, suggesting that these proteins can exist in a ternary complex (Figure 1b). They determined the biological significance of these interactions by generating *tpl bdl* double mutants. Strikingly, these double mutants, unlike *bdl* single mutants, formed hypocotyls, roots and cotyledon vasculature, suggesting that *tpl* can suppress both basal and apical *bdl* patterning defects [3]. This suppression can be traced back to embryogenesis, demonstrating that *tpl bdl*

double-mutant embryos form normal basal structures with restored auxin responsiveness as monitored by *DR5*. Conversely, while another, loss-of-function, allele of *bdl* does not display visible phenotypes, it enhances the frequency of severe *tpl* phenotypes at the permissive temperature (24°C) in the respective double mutant with *tpl*.

To corroborate these findings, Szemenyei *et al*. [3] carried out a series of innovative *in planta* experiments, which showed that TPL affects transcriptional repression by BDL. Moreover, expression of a chimeric protein consisting of the MP-interaction domain of BDL and the repression domain of TPL triggered *bdl* and *mp* phenotypes. Importantly, this chimeric protein was missing the BDL domain required for its auxin-mediated degradation, as well as the EAR motif of BDL, which mediates transcriptional repression of ARFs [15] and interaction with TPL (Figure 1b). Thus, the EAR motif of AUX/IAA proteins appears to repress transcription by recruiting TPL-family proteins and bringing them in close vicinity to ARFs.

Putting the picture together

How can the findings about the molecular interplay of these diverse players be reconciled with the various mutant phenotypes? A parsimonious explanation would be that they reflect region-specific variations in ARF activity. For instance, in the wild-type embryo, the polar auxin-transport machinery concentrates auxin at the basal pole of the embryo, leading to high ARF (here mainly MP) activity and thus root formation [6]. In contrast, in the apical regions of the embryo auxin concentration, and presumably ARF activity, is lower, resulting in formation of a shoot rather than a root. In *tpl* mutants, the maximal interference with TPL-like activity at restrictive temperatures might remove the repression of ARF activity to a level that overrides the normally insufficient apical auxin concentration, resulting in formation of root instead of shoot structures.

A caveat to this idea is the weak or absent apical *DR5* reporter expression in early *tpl* embryos [2]. However, this could reflect the temporal delay associated with reprogramming of the shoot or the fact that *DR5* is an artificial reporter of global auxin-induced transcription and may not always faithfully monitor activity of auxin target subsets. Global interference with the auxin pathway by the mutant protein tpl might not be necessary to initiate root formation. Rather, above-threshold expression of a few ARF-controlled master regulators of root formation, such as the *PLETHORA* genes [19], might suffice.

In *tpl mp* double mutants, the dominant-negative effects of the *tpl* mutation are limited by the absence of MP, particularly in the basal embryo, where this ARF is the limiting factor for root formation [8]. In the apical embryo, however, where other ARFs act redundantly with MP [20], *tpl* can still exert an effect, which explains the partial restoration of *mp* hypocotyl and cotyledon vasculature defects and the occurrence of apical roots in *tpl mp* double mutants.

So what about the *tpl* single-mutant phenotypes at more permissive temperatures? In these conditions, apical roots are rarely observed. Rather, the *tpl* seedlings increasingly display fused cotyledons [2]. This temperature-sensitive shift in *tpl* phenotypes could simply reflect a temperature-dependent decrease in auxin levels [21] and thus less stringent requirement for TPL activity. Therefore, the phenotypes of *tpl* at more permissive temperatures might reflect a quantitatively less severe ectopic activation of ARFs. This would suggest that an elevation, as well as a lack, of auxin signaling can result in apical patterning defects. This idea is supported by analyses that showed correlation of fused cotyledon frequency with increased auxin signaling [22]. Moreover, apical accumulation of auxin also results in cotyledon fusions [23,24]. An important distinction between the various genetic and physiological conditions that lead to aberrant cotyledon patterns is that in cases of diminished auxin signaling, the apical defects are accompanied by a reduction in vasculature (for example, *bdl*, *mp*) [8,14]. By contrast, in cases of elevated auxin signaling, vascularization remains intact or even increases (for example [22,23]). The *tpl* mutant falls into the latter class, because it does not display reduced vascular tissue and can even suppress reduced vasculature of *bdl* and *mp*.

Previous work has shown that gain-of-function phenotypes triggered by excess post-embryonic *MP* activity can be neutralized by the dominant *bdl* allele [20]. The recent work on TPL by Szemenyei *et al*. [3] underscores this inherently quantitative nature of auxin signaling and clearly demonstrates that quantitative shifts in ARF repression yield very disparate developmental readouts. The temperature-sensitive action of the original *tpl* allele is a marvelous tool to address this topic in more detail. Using a tool-kit of specifically and/or conditionally expressed dominant-negative TPL-family proteins, dominant-negative AUX/IAAs and various ARF mutants and overexpressors, it is now possible to uncover how the auxin pathway diverged quantitatively in specific contexts to give rise to the multitude of morphological and physiological responses controlled by this hormone.

Acknowledgements

We would like to thank N Geldner for comments on the manuscript, the Swiss National Science Foundation and the Canton de Vaud for supporting the research in our lab, and the Marie-Curie post-doctoral fellowship program for support of KSO.

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