

## Addendum

# The role of *DORNROESCHEN (DRN)* and *DRN-LIKE (DRNL)* in Arabidopsis embryonic patterning

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Appropriate embryonic patterning is amongst the most fundamental processes in plant development, necessary for the correct specification of root and shoot apical meristems which generate all post-germination organs of a plant. Many mutations have been characterized which disrupt embryonic pattern formation and many recent studies have focussed on the role of auxin in establishing apical-basal polarity. Our recent work has demonstrated the role of two redundant AP2 transcription factors, *DORNROESCHEN (DRN)* and *DORNROESCHEN-LIKE (DRNL)* in the control of embryo patterning, upstream of auxin perception and/or response and that *DRN* in turn, is regulated by auxin. We also suggest both genes are involved in the change from radial to bilateral symmetry in the globular embryo and are responsible for positional information of meristem-specific genes such as *STM*. The promiscuous interaction of DRN and DRNL proteins with the redundant family of class III HD-ZIP partners may represent a way by which embryonic cell specification can be controlled by combinations of transcription factor complexes, together with auxin.

## Embryonic Patterning is Under Redundant Control

The elaboration of an embryo from a fertilized zygote into a fully differentiated seed is achieved by processes of cell division and cell differentiation. In Arabidopsis, patterning events involving the initiation of apical-basal and radial axes and stem cell production. Differentiation ensues from a highly characteristic and reproducible pattern of cell divisions throughout embryogenesis, which has enabled the identification of mutants with aberrant patterns. Many mutants have been reported which mainly either affect the apical domain, giving rise to altered cotyledon morphogenesis, or the basal domain, resulting in root defects. The low penetrance of embryo phenotypes

resulting from single mutations within many gene families demonstrates that redundancy is an important feature of the control of embryo patterning. Dynamic changes in auxin concentration have been implicated in patterning processes including the establishment of apical-basal polarity and bilateral symmetry.<sup>1,2</sup> Correspondingly, patterning phenotypes arise from mutations in genes involved in auxin signalling or transport such as *PINOID*,<sup>3</sup> or within the *PIN* family.<sup>1,2</sup> Additionally, mutations in the auxin response factor *MONOPTEROS (MP)* or its IAA inhibitor *BODENLOS (BDL)*,<sup>4,5</sup> the *CUC* gene family,<sup>6-8</sup> or higher order mutations within the class III HD-ZIP family of transcription factors,<sup>9</sup> result in disrupted embryonic patterning and effects on polar auxin transport.<sup>10</sup>

## *DRN/DRNL* Redundantly Affect Apical/Basal Polarity Via Auxin

We recently showed that the *DORNROESCHEN (DRN)* and paralogous *DORNROESCHEN-LIKE (DRNL)* genes redundantly specify correct cotyledon development and cell divisions, particularly in the hypophysis region, implicating the AP2 class of transcription factors in embryonic patterning.<sup>11</sup> Both genes alter apical/basal polarity by causing cell division defects in the basal embryo domain or cotyledon defects in the apical domain. Since expression of both genes is confined to the apical region, non-cell autonomous functions may be inferred, in a similar way to *MP/BDL* function,<sup>12</sup> with auxin being one putative signal. Several lines of evidence currently implicate a role for *DRN* in auxin perception/response. Firstly, the expression of two markers for auxin concentration/response, *DR5::GFP* and *PINI::PINI-GFP* is altered in a *drn* mutant background, suggesting *DRN* functions upstream of auxin response/polar transport. Secondly, mono- or polycotyledonous *drn* and *drnl* mutants have altered leaf phyllotaxis, known to be dependent on local concentrations of auxin.<sup>13</sup> Additionally, *DRN* upstream and downstream regulatory sequences contain several canonical auxin response elements (AREs) specifically targeted by auxin response factors (ARFs),<sup>14</sup> (Fig. 1A), which we have shown to be functional by mutational analysis and responsible for auxin-driven *DRN* expression in different parts of the embryo (unpublished data), showing that *DRN* not only also has a function upstream of auxin, but also downstream. *DRNL* regulatory sequences also contain AREs analogous in position and number to those for *DRN* (Fig. 1A) and we postulate that these elements may also be functional. Since auxin is also synthesized at the tips of the developing cotyledons,<sup>2</sup> in a similar

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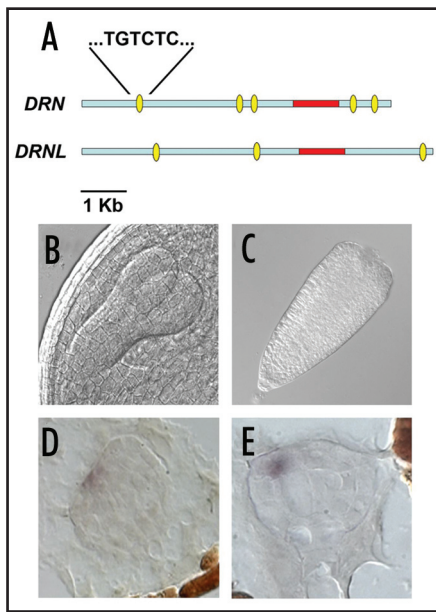


Figure 1. Comparison of the position of the TGTCC auxin response elements (ovals) within DRN and DRNL upstream and downstream genomic sequences. The DRN and DRNL coding regions are shown within this genomic region (A). Nomarski image of a wild type torpedo embryo (B) or a *drn-1 drnl-2* double mutant embryo at the same developmental stage, showing the absence of cotyledons (C). RNA in situ hybridization of an STM probe on wild type (D) or *drn-1 drnl-2* double mutant (E) transition stage embryos, showing the misexpression of STM in the mutant background.

domain to that of *DRN* expression, further work is needed to dissect the relevance of auxin transport from the regulation of biosynthesis.

### DRN/DRNL Affect Radial Symmetry

Cotyledon initiation demarcates the change from radial to bilateral symmetry during embryo development. Higher order mutants in auxin signalling or transport such as *pin1 pin3 pin4 pin7* and *pin1 pid* result in cotyledonless embryos, a phenotype shared by the *laterne* mutant, a result of mutation in both *PINOID* and *ENHANCER OF PINOID*,<sup>1,15,16</sup> underlining the importance of auxin during cotyledon 'anlage' initiation. *drn drnl* double mutants containing a strong *drnl* allele completely lack cotyledons, thereby abolishing radial symmetry (Fig. 1B and C). These double mutant embryos are a useful tool with which to study meristem development, since they initiate leaves, demonstrating firstly that *STM* function is not impaired, and secondly, that leaf initiation can be uncoupled from cotyledon formation. The study of other meristem-specific genes in *drn-1 drnl-2* double mutants, such as *SHOOTMERISTEMLESS* (*STM*), showed that although *STM* expression is not affected by loss of *DRN* and *DRNL* function, its spatial expression domain is altered (Fig. 1D and E), showing that *DRN* and *DRNL* provide positional information and control radial symmetry. Current work aims to identify the direct downstream targets of *DRN*, to elucidate the transcriptional cascades involved in its function.

### Redundancy May Reside in Higher Order Protein-Protein Complexes

*DRN* and *DRNL* proteins can physically interact in planta with *PHAVOLUTA* via their AP2 domains, and potentially with

all members of the class III HD-ZIP family, via a novel C-terminal PAS-like domain. This potential multiplicity of protein-protein interactions for *DRN* and *DRNL* and the redundant control of embryonic patterning in general raises the possibility that the robustness of embryo development may at least partially reside in the promiscuity of protein-protein interactions, with specificity being conferred by particular combinations of higher order transcription factor complexes subsequently affecting auxin fluxes and response. Disruption in *KANADI* or *HD-ZIP* class III gene families responsible for normal axis specification, compromises polar auxin transport via *PIN1*,<sup>10</sup> and supports this model. The identification of these higher order complexes and the transcriptional domains involved in their mutual function is a necessary goal in the challenge to dissect individual gene contributions within similar pathways.

### Recent Insights into DRNL Function

*DRNL* also been recently published as *SOB2*,<sup>17</sup> *ESR2*<sup>18</sup> and *BOLITA*,<sup>19</sup> suggesting diverse functions in light responses, shoot regeneration, and cell expansion and proliferation. Microarray analysis has shown that *DRNL* over-expression results in the altered regulation of many auxin-responsive transcripts, which strengthens a proposed function for *DRNL* in auxin signalling pathways,<sup>19</sup> and in the transcriptional regulation of *CUC1*.<sup>18</sup> Additionally, *DRNL* function has been implicated in stamen development,<sup>20</sup> suggesting a possible analogous function in organ boundary separation in flowers to that during cotyledon development.

### Perspectives

The phylogenetic study of *DRN* homologues from other species addresses evolutionary developmental questions: expression of the putative *DRN* orthologue in maize, *ZmDRN*, pre-patterns the presumptive scutellum domain at the late proembryo stage,<sup>21</sup> and lateral organ 'anlagen', as in Arabidopsis.<sup>22</sup> As the scutellum is a grass-specific organ, thought to be homologous to the cotyledon structure of dicots, this, together with the fact that putative *DRN* orthologues have also been isolated for rice,<sup>21</sup> suggests a conservation of *DRN* function across the monocot/dicot division.

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