Summary

Four basic ingredients of morphogenesis, oriented cell division and expansion, cell-cell communication and cell fate specification allow plant cells to develop into a wide variety of organismal architectures. A central question in plant biology is how these cellular processes are regulated and orchestrated. Here, we present the advantages of the early Arabidopsis embryo as a model for studying the control of morphogenesis. All ingredients of morphogenesis converge during embryogenesis, and the highly predictable nature of embryo development offers unprecedented opportunities for understanding their regulation in time and space. In this review we describe the morphogenetic principles underlying embryo patterning and discuss recent advances in their regulation. Morphogenesis is under tight transcriptional control and most genes that were identified as important regulators of embryo patterning encode transcription factors or components of signaling pathways. There exists, therefore, a large gap between the transcriptional control of embryo morphogenesis and the cellular execution. We describe the first such connections, and propose future directions that should help bridge this gap and generate comprehensive understanding of the control of morphogenesis.

I. Ingredients of plant morphogenesis

Morphogenesis occurs throughout plant life, and generates the enormous variety of shapes observed in the plant kingdom. No matter how complex the eventual plant morphology, whether a structurally simple moss or a highly branched tree, a small set of cellular processes underlie all shapes. Hence, an understanding how diversity in plant shapes, structures and functions is controlled requires knowledge of the basic cellular principles underlying morphogenesis. Among these are cell division, the process where a cell divides into two daughter cells, both with the same properties (symmetric) or with different properties (asymmetric) (both orientation and rate of cell division strongly contribute to morphogenesis) and (directional) cell expansion, the process whereby a cell expands its volume in either a random or a directional fashion. This occurs primarily through turgor pressure from the vacuole, guided by the cell wall (Sanchez-Rodriguez et al., 2010). These two processes (division and expansion) are cell-intrinsic features that define shape and growth direction. As such properties are uniquely controlled in different cell types, a
third important component of morphogenesis is cell fate specification. Finally, considering its vital importance for plant morphogenesis, we consider a fourth key process that coordinates cellular decisions in time and space: cell–cell communication (both short- and long-range). Short-range cell–cell communication mostly occurs using small signal molecules that diffuse either through the cell membrane or channels or are secreted to the apoplast and recognized by membrane receptors in adjacent cells (Nakajima et al., 2001; Hirakawa et al., 2008). Long-range communication is typically established by the use of hormones that work as either a ligand for membrane receptors or as an active compound within the cell (Ubeda-Tomas et al., 2012).

Whereas during animal development, cell migration plays a key role in morphogenesis, for example in gastrulation (Lim & Thiery, 2012), cell migration does not occur in plants, because of the presence of rigid cell walls. Since morphogenesis is strongly genetically controlled, development can be viewed as the sum of the transcriptional control of individual cell properties, combined with cell–cell signaling that connects cells. In this review, we discuss the use of the early embryo as a model that is excellently suited to study these processes and their interconnections. Early embryogenesis is interesting, not only because morphogenesis occurs in a very controlled manner (see below), but also because the tissues, the cell types that populate them, and the stem cell systems that maintain them are specified de novo.

During plant development, several niches of stem cells (see Box 1) are established. These niches, or meristems, are established as early as the globular stage of Arabidopsis embryo development, are maintained throughout the life of a plant and function to establish the new tissues and organs (Weigel & Jurgens, 2002). Although much has been learnt about meristem function and maintenance from studies of postembryonic development (Liu et al., 2009; Terpstra & Heidstra, 2009), the exact processes underlying the initiation of stem cells and their meristems are still poorly understood. One of the many challenges of developmental biology is to separate genetic control from environmental control of the tissue that is studied. As both can play a major role in the development of an organism, the ability to separate these two factors is of great importance. The challenge in doing this lies mainly in the fact that both factors are often interdependent and respond to each other (Hsu & Harmer, 2012). Particularly because organogenesis is a highly plastic process on which many environmental factors converge, postembryonic development is not entirely predictable at the cellular level. The early embryo, by contrast, is resilient to environmental influences, as the same body pattern is generated under various conditions. Despite its simplicity (Figs 1, 2), the few first days of plant life produce an embryo in which all ingredients of morphogenesis combine to generate the main tissues, cell types and meristems. Although different plant species can differ greatly in the final product of embryo development (Johri et al., 1992), the same basic principles apply, as in all cases a species-specific robust pattern with comparable pattern elements is formed. Since most research has been performed on the development of the Arabidopsis embryo, we will mainly focus on this model in this review. We will discuss the key steps in embryonic pattern formation and recent insights into their regulation. We will consider future directions and challenges in revealing the molecular and cellular basis for plant morphogenesis using the embryo as a model.

**II. Arabidopsis embryo development: an amalgamation of morphogenetic processes**

There have been many reports and reviews describing the sequence of divisions during Arabidopsis embryogenesis (De Smet et al., 2010; Peris et al., 2010; Nodine et al., 2011). Here, we would like to take a slightly different approach to
axis, the radial axis. The outermost cells are the so-called protodermal cells, which will undergo several rounds of symmetric cell divisions and later establish the epidermal tissue. During the following step to the early-globular stage, the first internal tissues are specified. Through a round of asymmetric cell divisions, the inner cells form the precursors of the vascular and ground tissue. Also during this step, the hypophysis is specified from the most apical suspensor cell, which then protrudes (and is incorporated) into the embryo. The steps taken to form the late-globular embryo may be regarded as some of the most essential steps during the development of the embryo. During the transition from the early- to the late-globular stage, the hypophysis divides asymmetrically to form the small apical lens-shaped cell and the larger basal cell. These are known as the root organizer (in later stages called the quiescent center (QC)) and columella stem cells, respectively. Cell–cell signaling has been shown to be crucial for hypophysis specification to occur properly (Hamann et al., 1999; Schlereth et al., 2010). Also during this stage, the stem cells for the vasculature and ground tissue are specified and divide once to form apical daughter cells that will further divide and differentiate. In later stages, more cell expansion and differentiation take place, together with the specification of the shoot apical meristem cells.

As will be detailed below, all the earlier-mentioned processes are under tight genetic control, as mutations in key components lead to specific defects. Furthermore, the different cell identities as described here are often marked by specific gene expression markers (Figs 2, 3, 5).

III. Regulation of morphogenetic processes during embryogenesis

Even though embryogenesis is a continuous process, and all patterning processes depend on appropriate prior patterning, we will here consider the regulation of the critical morphogenetic processes during embryo development individually, with an emphasis on recent findings that have shed light on the mechanisms involved.

1. Development before and directly after fertilization

During the very first stages of embryo development, the zygote, right after fertilization, loses its polarity, which is later re-established. To our knowledge, there have so far been only three reports showing the depolarization and subsequent repolarization of the zygote after fertilization (Christensen et al., 2002; Faure et al., 2002; Ueda et al., 2011), and in all cases the phenomenon has been descriptively reported. Therefore, it remains unknown why this happens, what mechanisms are involved in the process and the precise function of the depolarization. Before fertilization of the egg cell, a high degree of polarity can be observed, but the factors that are important for this polarity are not well understood. One can speculate about the meaning of this polarity and, perhaps more importantly, the meaning of the depolarization. It is conceivable that during development of the female gametophyte, the cells surrounding the egg cell produce signals that determine egg cell polarity and that, after fertilization, these signals
are no longer produced or available, resulting in depolarization of the egg cell. It is also possible that the subsequent availability of factors required for repolarization determines whether the first division of the zygote occurs symmetrically or asymmetrically. Some plant species have been shown to lack this characteristic asymmetric division seen in Arabidopsis (Johri et al., 1992). In Arabidopsis, the WRKY2 gene has been shown to be a factor required for repolarization of the egg cell after fertilization (Ueda et al., 2011). A large-scale study combining transcriptomic and physiological approaches comparing different plant species could potentially unravel the mechanisms that underlie the process of depolarization and subsequent repolarization and perhaps shed light on the biological function of these processes. In Arabidopsis, the WRKY2 gene, encoding a zinc-finger transcription factor, was the first and so far the only genetic regulator of zygote repolarization to be identified (Ueda et al., 2011; see Fig. 3). In the absence of WRKY2 activity, the polarity of the egg cell is lost after fertilization, similar to the wildtype. In contrast to the wildtype zygote, polarity is not re-established and the subsequent division seems to occur symmetrically rather than asymmetrically (based on cytoplasm density and the presence of vacuoles). Later divisions of the suspensor cells also occur erroneously, as the suspensor cells are very small and sometimes divide periclinally, resembling embryonic cells (Ueda et al., 2011). This indicates that WRKY2 also has a role in specification or maintenance of the suspensor identity. The role of WRKY2 in suspensor identity is most likely through regulation of WOX8/9 expression in the zygote. In the wkry2 mutant background, WOX8 is severely down-regulated in the zygote and expression of the embryo-specific WOX2 gene is expanded to erroneously dividing cells of the suspensor (Ueda et al., 2011), which further indicates that these cells attain an embryo-like fate. Although WOX8 expression is greatly reduced in the zygote, this expression is regained by the two-cell stage, showing that other factors also control WOX8 expression and this is most likely the reason why the
**wrky2** mutant produces relatively normal mature embryos. In addition to regulation of WOX8 and WOX9 expression in the zygote, there seems to be another, as yet unknown, factor regulated by WRKY2, as the wox8 wox9 double mutant does not show a phenotype until after the one-cell stage (see Box 2), and expression of WOX8 in the wrky2 background cannot fully rescue the phenotype (Ueda et al., 2011). This suggests that there are factors yet to be discovered that play a role in the process of zygote polarity and the establishment of cell types in this early stage.

A further step is elongation of the zygote. Several factors have been found that influence zygote elongation and one of the main pathways is the so-called YODA (YDA)-signaling pathway (see Fig. 3). YDA encodes a mitogen-activated protein kinase kinase (MAPKKK) that was first isolated through a mutant screen for defects in suspensor development (Lukowitz et al., 2004). In this screen, two other genes (SHORT SUSPENSOR (SSP) and GROUNDED (GRD)) were found to have similar phenotypes and, indeed, these were found to act in the YDA-signaling pathway. The YDA gene is ubiquitously expressed, but the effect on elongation seemed to be restricted to a specific developmental cue, as no significant effect on elongation was found in other cells (Lukowitz et al., 2004). YDA does have a broader function, perhaps unrelated to elongation, as it was shown to regulate stomatal development (Bergmann et al., 2004). In the yda mutant, elongation of the zygote is severely reduced, resulting in a very short suspensor and often erroneous divisions in the suspensor at later stages. Interestingly, a hyperactive variant of YDA has an almost opposite effect on embryo development, resulting in excessive elongation of the suspensor, which was often found to contain more cells than normal (Lukowitz et al., 2004). More recently, the YDA-signaling pathway was found to be controlled by a member of the interleukin-1 receptor-associated kinase (IRAK)/Pelle-like kinase family of receptor-like kinases, called the SHORT SUSPENSOR (SSP: Bayer et al., 2009). Remarkably, this was one of the genes retrieved from the same screening that resulted in isolation of the YDA gene (Lukowitz et al., 2004). MAP kinase signaling pathways are, in both animals, and plants, often under the control of receptor-like kinases that are activated by an external signal and this occurs on a protein level is as yet unknown, although GRD/RKD4 is involved in asymmetric division of the zygote (Schauser et al., 2005), which implies a role for these proteins in DNA binding and regulation of transcription. Indeed, Waki et al. (2011) found that GRD/RKD4 could trigger embryo-specific gene expression upon overexpression, in some cases even resulting in the induction of somatic embryogenesis. Phenotypically, the grd1 rkd4 mutants resemble the yda and sp mutant, with defects in zygote elongation and suspensor formation, and GRD/RKD4 was shown to have a genetic interaction with the YDA-signaling pathway (see Fig. 3; Jeong et al., 2011). Although it is not a direct target of YDA signaling, as overexpression of GRD/RKD4 in a yda background does not suppress the yda phenotype, it is required for a functional YDA cascade, as the hyperactive yda mutant does not have an effect on the grd1 rkd4 phenotype (Jeong et al., 2011). How this interaction occurs on a protein level is as yet unknown, although GRD/RKD4 could be regulating the expression of target genes active in the YDA-signaling pathway, either with the cooperation of the YDA cascade or completely independently. In addition to its function in the YDA-signaling pathway, GRD/RKD4 was found to act cooperatively with WOX8 and WOX9 in establishing embryo polarity (see Fig. 3). Embryos lacking all three genes stop developing very early on and never progress beyond the one-cell stage (Jeong et al., 2011). This enhancement of both phenotypes (Box 2) indicates that there is a synergistic relationship between the WOX and the YDA pathway, strengthening both their roles in the development of the plant embryo.

**2. Regulation of the two-cell stage to the dermatogen stage**

Many of the remaining questions concerning regulation of morphogenetic processes arise in relation to development from the two-cell stage to the dermatogen stage. During these stages, the
basis of many cell types is established, through the process of axis and pattern formation. Both the apicobasal and radial axes are formed at this time, but how the formation of these two most important axes is regulated is still largely unknown. Although some important advances have been made, as will be discussed here (see also Fig. 4), many gaps still remain.

Box 2 WOX genes

WOX8/9 are part of the family of WUSCHEL RELATED HOMEOBOX (WOX) genes, which have been shown to be regulators of a whole range of developmental processes, including embryogenesis (van der Graaff et al., 2009). The founder of this gene family, WUSCHEL, regulates maintenance of the shoot apical meristem (Laux et al., 1996). Interestingly, the wus mutant is able to produce new meristems, but these are not maintained and soon terminate their growth. WUS expression can be found in the organizing center of the shoot apical meristem (Mayer et al., 1998), similar to a distant relative, WOX5, which is found in the quiescent center (QC) cells of the root meristem and is important for root meristem maintenance (Sarkar et al., 2007). Strikingly, the wus phenotype can be complemented by expressing WOX5 in the WUS domain (Sarkar et al., 2007), indicating that, although not closely related, these factors share similar functions in the maintenance of meristems. This is also consistent with phylogenetic data that shows there is a single common ancestor for all the WOX genes in green algae and a single WUS/WOX5 homolog found in gymnosperms (Nardmann et al., 2009), indicating that a subfunctionalization event occurred that kept meristem maintenance function in both genes, but diverged them into two different expression domains. The role of WOX genes in early embryogenesis becomes most apparent in the interplay between the WOX2 and WOX8/9 genes. Initially, these genes are coexpressed in the undivided zygote but they become restricted to the apical (WOX2) and basal (WOX8/9) cell after the first division, marking the separate cell lineages and also showing the asymmetric division on a molecular level (Haecker et al., 2004). Single mutants in WOX8 and WOX9 do not show any apparent morphological difference to the wildtype embryos, but when both WOX8 and WOX9 function is disrupted, several developmental defects can be observed (Breuninger et al., 2008). Up until the one-cell stage of development, the embryos resemble the wildtype phenotype, although subsequently the apical cell divides horizontally instead of vertically and the basal cells become enlarged and show aberrant division planes. These mutants also show defects on a molecular level, as they seem to lose the expression of several basal (e.g. WOX5) and apical (e.g. ZWILLE) markers and show an increased auxin response demonstrated by more, and ectopic, DR5 expression. In addition to this, the authors were able to show that WOX8 and WOX9 are positive regulators of the WOX2 expression in the embryo (Breuninger et al., 2008). This indicates that the specification of the basal cell lineage is under the control of WOX8 and WOX9 and that this specification is very important, not only for the development of the basal cell lineage, but also for the apical cell lineage.

In recent years, it has become increasingly clear that several patterning steps in the embryo strongly depend on the activity of the hormone auxin. The auxin signaling pathway is a relatively simple and short one that is involved in many developmental processes, including root growth and development, flowering, apical dominance, formation and organization of vascular tissue, fruit growth and development, and embryogenesis (Stewart & Nemhauser, 2010). It consists of four main components: the SCFTIR1/AFB ubiquitin ligase complex (Ruegger et al., 1998), Aux/IAA inhibitor proteins (Reed, 2001), DNA-binding AUXIN RESPONSE FACTORS (ARFs; Guilfoyle & Hagen, 2007) and the phytohormone auxin. At low auxin concentrations, the Aux/IAA proteins bind to the ARFs and inhibit their function in regulating genes (Tiwari et al., 2001, 2003). When auxin concentrations increase, auxin binds to the TIR1/AFB1-5 subunit of the SCFTIR1/AFB ubiquitin-ligase complex, increasing the affinity of this complex to the Aux/IAA proteins (Gray et al., 2001; Dharmasiri et al., 2005a; Kepinski & Leyser, 2005). Upon ubiquitination, the Aux/IAA proteins are degraded by the 26S proteasome, releasing the ARFs from their inhibition and leaving them to perform their function as transcriptional regulators (reviewed by Lokerse & Weijers, 2009). Several Aux/IAAs were shown to act in part by recruiting the corepressor TOPLESS (TPL; Szemenyei et al., 2008).

The amount of auxin present in a cell is tightly controlled. Owing to the relatively low pH in the apoplast, auxin becomes protonated and is able to diffuse passively through the cell membrane into the cell, where the higher pH results in deprotonation and blockage of passive diffusion through the cell membrane (Rubery & Sheldrake, 1973). Export of auxin occurs actively through the PIN-FORMED protein family of auxin exporters (Grunewald & Friml, 2010). These often polar- and membrane-localized proteins facilitate directional transport of auxin, and thus allow local maxima to be generated (Grunewald & Friml, 2010). These auxin maxima have been shown to be important for maintenance and also the establishment of meristems. One of the main questions in auxin research is how a small molecule like auxin, triggering such a short and seemingly simple signaling pathway, can regulate so many different developmental processes and how the specificity of this pathway is regulated. The proteins involved in the auxin signaling are all part of larger protein families. The TIR1/AFB, Aux/IAA and ARF families consist of six, 29 and 23 members, respectively (Reed, 2001; Dharmasiri et al., 2005b; Guilfoyle & Hagen, 2007), and different combinations of these members, through differences in availability and affinity, could account for some of the specificity in the signaling. Interestingly, Rademacher et al. (2011) showed there is a pre-pattern of ARF gene expression in the embryo, which could account for, or may at least contribute to, specific auxin responses in different cell types. As different cell types all have a distinct set of ARFs, they will respond differently to the same stimulus, which will result in a different developmental output. Conversely, cells that are supposedly the same, sharing the same set of ARFs, will respond in the same way. It is unclear how the pre-pattern of ARF expression is itself established, but this mechanism at least involves the SSP gene (Rademacher et al., 2012). In support of the importance of the ARF pre-pattern, several mutants were identified where multiple ARFs, in the suspensor, were either knocked out or constitutively repressed using a stable Aux/IAA protein. Both these approaches lead to excessive proliferation of the suspensor, loss of suspensor-specific gene expression (e.g. IAA30) and a gain of embryo-specific gene expression (e.g. 2013 The Authors
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MONOPTEROS; SHOOT MERISTEMLESS, KNOLLE, and WUS); occasionally, suppression of the auxin signaling in the suspensor even leads to twin embryo phenotypes (Rademacher et al., 2011, 2012). This finding suggests that the ability of suspensor cells to develop into an embryo, which was recognized a long time ago (Schwartz et al., 1994), is actively suppressed by the signaling molecule auxin.

As becomes apparent from the information in the previous paragraph, maintaining suspensor identity is important for normal embryo development. The juxtaposition of two distinct cell identities (embryo vs suspensor), by definition, creates a boundary domain. Recently, the HANABA TARANU (HAN) gene was found to be maintaining this basal boundary in the early stages of embryogenesis (Fig. 4; Nayw et al., 2010). This GATA transcriptional repressor (Zhang et al., 2013) was shown to regulate transcription of several genes in the lower tier of the embryo and, upon disruption of its function, their expression domains were expanded to more apical (e.g. SUCROSE TRANSPORTER3, WOX5, SHORT-ROOT) or lateral regions (e.g. PLETHORA4) (Nawy et al., 2010). Active auxin signaling, usually seen in the hypophysis descendant cells (Friml et al., 2003; Weijers et al., 2006), also shifted upwards (Nawy et al., 2010), which may be the consequence of a change in PIN1 and PIN7 expression. Where PIN1 is normally localized in the basal membrane of the inner cells of the embryo (Steinmann et al., 1999), directing auxin towards the base and the hypophysis, in the case of the han mutant, PIN1 accumulates mainly in the apical cells (Nawy et al., 2010).

Similarly, in the wildtype, PIN7 is localized in the apical membrane of the suspensor, marking the boundary between the suspensor and the embryo (Friml et al., 2003), but in the han mutant PIN7 is found mainly in the apical membrane of the basal cells of the embryo. Interestingly, the absence of a functional HAN protein results in an initially rootless embryo, though the root is later reinitiated and han seedlings do have a main root (Nawy et al., 2010). These findings underline the importance of this fate boundary, although the precise molecular and cellular definitions of this domain are unclear.

In addition to its function in zygote polarity specification (see Box 2), WOX2 has also been shown to act redundantly with the closely related WOX1 and WOX3 genes in the regulation of the protoderm-forming cell divisions in the upper tier (see Fig. 4; Breuninger et al., 2008). In wox1/2/3 mutant embryos, division of cells in the upper tier often occurs periclinally, which occasionally results in seedlings with one cotyledon or ‘rod-shaped’ seedlings (Breuninger et al., 2008). Although this indicates the mechanism regulating the upper tier, it is still unclear how this change in division plane is regulated for the lower tier (Fig. 4). It is possible that WOX9 could play a role in this, as it has a complementary expression pattern to WOX2. A phenotype could be masked by the more extreme phenotype of the wox8/9 double mutant (Breuninger et al., 2008), although this remains to be investigated. The mechanisms underlying the change of division planes in the two-cell and octant stages also remain elusive to date.

3. Globular stage of development – stem cell and tissue specification

A crucial step in development is the globular stage. It is during this stage (from early- to late-globular) that the root tissues and their stem cells are specified (see Fig. 5). These are also the stages where most mutants show phenotypes (Lloyd & Meinke, 2012). Perhaps one of the most well-known factors to play a role in specification of root cell types is the AUXIN RESPONSE FACTORS/ MONOPTEROS (MP) gene, as it has been shown to be a key regulator in hypophysis specification and specification of other cells that form the embryonic root (Berleth & Jurgens, 1993; Hardtke & Berleth, 1998; Weijers et al., 2006). Mutations in the MP gene result in mis specification of the hypophysis, which leads the cells in the basal tier to divide erroneously, eventually resulting in rootless seedlings (Berleth & Jurgens, 1993; Hardtke & Berleth, 1998). As with all members of the ARF family, auxin and the Aux/IAA family of transcriptional repressors regulate MP function, and Schlereth et al. (2010) recently revealed the mechanism by which MP regulates hypophysis specification. Using an inducible version of the Aux/IAA protein IAA12/BDL that cannot be degraded and induces an mp-like phenotype (Hamann et al., 1999; Hamann et al., 2002), MP function was briefly inhibited. After applying a microarray approach, they were able reveal a set of differentially expressed genes. Further characterization of target genes revealed several direct TARGETOFMONOPTEROS (TMO) genes. Two of these, TMO5 and TMO7, encode basic helix–loop–helix (bHLH) transcription factors and both are only transcribed in the basal inner cells of the proembryo (Schlereth et al., 2010; Fig. 5). Interestingly, the TMO7 protein was shown to move from its transcribed region to the hypophysis. By addition of either a single or triple GREEN FLUORESCENT PROTEIN (GFP) molecule to the TMO7 protein, the authors were able to show that this movement is size-dependent and is also required for specification of the hypophysis. TMO7 expression in the suspensor was found to partly rescue the mp phenotype (Schlereth et al., 2010). Recently, TMO5 was shown to form a dimer with another member of the bHLH family, LONESOME HIGHWAY (Ohashi-Ito & Bergmann, 2007), and as a dimer trigger oriented periclinal divisions during the
development of the vascular tissue in the early embryo (De Rybel et al., 2013). While TMO5 and LHW are critical for the divisions that 'make' the vascular tissue, little is known about what initially specifies the tissue. This is one of the major outstanding questions, and genetic approaches thus far have not revealed the critical components for embryonic vascular or ground tissue specification.

In addition to specification of stem cells and tissues, a different layer of information is generated at this stage of embryogenesis, namely that of regional identity. Cotyledons, shoot apical meristem and root all express unique sets of genes and have distinct organ identities (Long et al., 1996; Long & Barton, 1998; Aida et al., 2004). The PLETHORA (PLT) transcription factors (Aida et al., 2004) seem to play an important role in specification of regional identity at this stage of embryogenesis. PLT1 and PLT2 are key regulators of root meristem maintenance and root 'fate' (Aida et al., 2004). The PLETHORA (PLT) transcription factors (Aida et al., 2004; Galinha et al., 2007). Removal of several PLT genes leads to rootless embryos (Galinha et al., 2007), while misexpression can induce the formation of ectopic root structures (Aida et al., 2004).

It was recently shown that exclusion of PLT gene expression from the apical embryo domain is required for shoot formation (Smith & Long, 2010). PLT expression was found to be inhibited by CLASS III HOMEODOMAIN-LEUCINE ZIPPER genes (HD-ZIP III; i.e. PHABULOSA, PHAVOLUTA, REVOLUTA, INCURVATA4 and ARABIDOPSIS THALIANA HOMEobox-8), which are expressed in the apical part of the embryo and regulated by MICRO-RNA165/166 (Emery et al., 2003; Mallory et al., 2005; Smith & Long, 2010; Miyashima et al., 2013). Interestingly, ectopically expressing a microRNA-resistant version of any of the HD-ZIP III genes under the PLT2 promoter results in the opposite phenotype as found for the overexpression lines of the PLT1s: a shoot in place of a root (Smith & Long, 2010). This shows that root and shoot fates are mutually exclusive, and that part of the root or shoot program entails suppression of the alternative fate (see Fig. 5).

As shown by the regulation of the HD-ZIP III genes in the apical fate regulation, microRNAs control development of the Arabidopsis embryo. Indeed, systematic expression analysis of the MICRO-RNA165/166 gene family revealed an extended potential for regulation of HD-ZIP III accumulation by these factors (Miyashima et al., 2013). As microRNAs are involved in a wide range of processes in all organisms that carry them (Furuta et al., 2012), there is the distinct possibility that there are more, as yet unidentified, roles for these regulators in early plant embryogenesis. Such functions are easily missed in genetic screens, as only some mutations will alter microRNA efficiency (Schwab et al., 2005), and often the microRNAs are represented by gene families (Miyashima et al., 2013). Evidence for such novel functions in embryogenesis comes from analysis of the microRNA biogenesis mutant dicer-like1 (dcl1). Many developmental processes are disturbed in dcl1 mutant embryos, including the loss of expression of several cell-lineage markers, such as WOX2, -5 and -8, and expansion of the protoderm markers MERISTEM LAYER1 (ATML1) and PROTODERMAL FACTOR1 to the suspensor (Nodine & Bartel, 2010). By sequencing the transcriptome of dcl1 mutant embryos, it was shown that many late-embryogenesis genes are precociously active. This can be explained to a large degree by unchecked activity of SQUAMOSA PROMOTER BINDING PROTEIN-LIKE10 (SPL10) and SPL11 are derepressed by the down-regulation of miR156, which prevents maturation in earlier stages. In the late-globular stage, a dimer of TMO5 (whose expression is driven by MP) and LHW regulates the development of vascular tissue. The colors indicate the expression domains of different genes.

As indicated, this stage of embryogenesis can be considered the nexus where tissues, cell types, regions and stem cells are specified. The latter component, stem cell specification, remains a relatively unexplored area. Although the existence of cells with stem cell properties (see also Box 1) in plant meristems and their importance for sustaining growth are undisputed, there is no clear operational or mechanistic definition of such cells. While stem cells in mice or humans can be functionally defined by transplantation or clone formation assays (Huch et al., 2013), such experiments are extremely challenging in plants, as cells are usually respecified when they find themselves in a different context (Scheres, 2001). While molecular criteria exist for some animal stem cells (Barker et al., 2007), no markers for stem cells exist in plants. Finally, while stem cell-like properties can be induced by a mix of proteins in mice and humans (Takahashi & Yamanaka, 2006; Yu et al., 2007), similar factors have not been reported in plants. Therefore, definition of stem cells remains
somewhat difficult in many plant tissues, except for those such as the columella root cap, where a single (stem) cell layer separates the differentiated cells from the organizing center (Scheres, 2007). These difficulties extend to the problem of when exactly stem cells are first specified in the embryo. From lineage-tracing experiments (Scheres et al., 1994), it is clear that the cells that act as long-term stem cells in the root meristem can be traced to the basal tier cells of the globular stage embryo. Indeed, genes that mark the presumptive stem cell zone in the postembryonic root meristem are first activated at this stage of development (De Rybel et al., 2013). Hence, we anticipate that the globular stage embryo is a promising, yet largely unexplored model for identifying elusive stem cell factors in plants.

IV. Genes to shape – cellular execution of transcriptional instructions

All genes described in this review as key regulators of morphogenetic processes during early embryogenesis encode transcription factors, (receptor) kinases, hormones and microRNAs that, in turn, regulate transcription factors. These factors can collectively account for two key processes as defined earlier: cell specification and cell–cell communication. Yet, as discussed, the shape-defining morphogenetic drivers in plants are oriented and/or asymmetric cell division and (directional) cell expansion. A pressing question is therefore how unique cell identities, established as distinct cellular transcriptomes, are translated to reprogramming of the mechanisms underlying these two morphogenetic drivers. This is a challenge that has so far not been addressed in embryos, but, as outlined earlier, the embryo offers a compact and predictable model to study this problem. The key question is which genes need to be regulated for cell division rate or plane, or cell expansion rate or direction, to change. Many generic components in these processes have been identified, and these include many regulators of actin or microtubule cytoskeleton (Yang, 2008), cell wall composition or extensibility (Roppolo & Geldner, 2012), cell cycle (Komaki & Sugimoto, 2012), cell plate orientation (Torres-Ruiz & Jurgens, 1994) and membrane trafficking (Lukowitz et al., 1996). Without exception, interfering with the function of these components causes dramatic defects at both the cell and tissue levels (Turner & Somerville, 1997; Bao et al., 2001; Schnittger et al., 2003; Ambrose et al., 2007), but rarely are such defects limited to individual cells, which complicates connecting these factors to regulators of pattern formation. A few recent papers have started bridging the gap between pattern regulators and cell biological functions. The transcription factor SHORT-ROOT (SHR) controls asymmetric division of the initial cell that gives rise to endodermis and cortex in the postembryonic root (Helariutta et al., 2000), and in addition helps in specifying endodermis identity (Cui et al., 2007). Through transcriptomics approaches, a CyclinD6 gene was recently identified as a direct SHR target that is required for triggering the asymmetric (formative) division in this initial cell (Sozzani et al., 2010). This is a direct connection between a patterning factor and the machinery that executes cell division. Furthermore, it was recently shown that the PLT2 transcription factor directly regulates expression of the microtubule-binding proteins MAP65-1 and MAP652, which regulate division plane rotation through the CLASP protein in the lateral root cap. Manipulating PLT2, MAP65 or CLASP components causes switching between periclinal and anticlinal division orientations (Dhonukse et al., 2012). A very interesting challenge will be to extend such findings to the embryo, and more generally link patterning to morphogenesis.

V. Concluding remarks

During plant development, several morphogenetic processes need to be tightly regulated, in order to ensure proper establishment of specific cell types and tissues. In this review we have highlighted what we consider to be the most important morphogenetic processes involved in the development of the Arabidopsis embryo and how these processes are regulated. Although much has been invested in finding new factors that are important in the regulation of these processes, and indeed several pathways have been identified, many questions still remain to be answered. Owing to the inherent difficulties associated with the small size of embryos in Arabidopsis, and their encapsulation in seeds within fruits, methods other than genetics have not been explored to their full potential. With the methodological and technological advances of recent years, the way is now clear to address important questions in this developmental system. We anticipate that the coming years will bring answers to the following questions:

- What marks and defines individual cell types?

  The use of single-cell or cell type-specific transcript profiling approaches has the potential to provide genome-wide views on molecular differentiation of cells. Such analysis will also be helpful to identify cell-specific functions and pathways. New techniques, such as Isolation of Nuclei TAGged in specific Cell Types (INTACT; Deal & Henikoff, 2010) should help in obtaining transcriptomes at cellular resolution from embryos, through development.

- How do key morphogenesis regulators exert their function?

  Identification of the cellular processes that are targeted by the transcriptional regulators that control morphogenesis will be an essential step. Transcriptional targets are being identified for transcription factors, for example using chromatin immunoprecipitation coupled to next-generation sequencing (Kaufmann et al., 2010).

- What are the regulatory mechanisms by which key morphogenesis regulators act?

  While most factors that control aspects of morphogenesis in embryos are being regarded in isolation, most proteins act in larger complexes. A key challenge will be to identify such active complexes, as this will help to single out and localize the active fraction of regulators. Advances in mass spectrometry allow such approaches to be applied to plant embryos (De Rybel et al., 2013).

- How is life in four dimensions (4D)?

  Most of what we know about embryo development is derived from studies in which two-dimensional (2D) images are used as a basis for interpretation. Two key challenges for researchers are to generate understanding of the 3D arrangement of cells in embryos, and to observe embryos live in 4D imaging. While the
latter will require novel or dedicated microscopy approaches that are compatible with vitality, the first could be achieved by applying conventional confocal imaging and 3D reconstruction to fixed embryos (De Rybel et al., 2013).

• Beyond weeds – how have embryo morphogenesis mechanisms evolved?

Several of the processes that sculpt the embryo are evolutionary old. Likewise, as the defining feature of the large embryophyte lineage, the embryo is itself also an ‘old’ structure. An important question is whether the regulatory mechanisms that drive morphogenesis in the Arabidopsis embryo date to the origin of the embryophytes. The increasing availability of plant genome sequences and development of more basal land plant models (Bowman, 2012) should help in addressing this question.

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