CHAPTER ONE

Genetic and Epigenetic Controls of Plant Regeneration

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Abstract

Plants have evolved powerful regeneration abilities to recover from damage. Studies on plant regeneration are of high significance as the underlying mechanisms of plant regeneration are not only linking to the fundamental researches in many fields but also to the development of widely used plant biotechnology. Higher plants show three main types of regeneration: tissue regeneration, *de novo* organogenesis, and somatic embryogenesis. In this review, we summarize recent research on plant regeneration, mainly focusing on *Arabidopsis thaliana* and moss. New data suggest that plant hormones trigger regeneration and that several key transcription factors respond to hormone signals to determine cell-fate transition. Cell-fate transition requires genome-wide changes in gene expression, which are regulated via epigenetic pathways. Certain epigenetic factors may be recruited by transcription factors to relocate

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to new loci and regulate gene expression. Cross talk among hormone signaling, transcription factors, and epigenetic factors is involved in different types of plant regeneration, suggesting that elegant and complex regulatory mechanisms control which type of regeneration is triggered in plants under different circumstances. Since regeneration is initiated by wounding, identification of the wound signal is an important objective for future research.

ABBREVIATIONS

2,4-D 2,4-dichlorophenoxyacetic acid
2-IP 6-(γ,γ-dimethylallylamino) purine
ABA abscisic acid
AGL15 AGAMOUS-LIKE15
ALF4 ABERRANT LATERAL ROOT FORMATION 4
AP2 APETALA2
BBM BABY BOOM
CDKA cyclin-dependent kinase A
CIM callus-inducing medium
CLF CURLY LEAF
CUC2 CUP-SHAPED COTYLEDON2
EMF2 EMBRYONIC FLOWER2
EMK EMBRYOMAKER
ES cell embryonic stem cell
ESR1 ENHANCER OF SHOOT REGENERATION1
EZ elongation zone
FUS3 FUSCA3
GA gibberellin
H2Aub histone H2A ubiquitination
H3K27me3 histone H3 lysine 27 trimethylation
iPS cell induced pluripotent stem cell
JA jasmonic acid
KYP KRYPTONITE
LBD LATERAL ORGAN BOUNDARIES DOMAIN
LEC LEAFY COTYLEDON
LHP1 LIKE HETEROCHROMATIN PROTEIN1
MET1 METHYLTRANSFERASE1
NAC NAM, ATAF1,2, and CUC2
NPA naphthylphthalamic acid
OC organizing center
PcG Polycomb group
PIN PIN-FORMED
PKL PICKLE
PKR2 PICKLE-RELATED2
PLT PLETHORA
PRC Polycomb repressive complex
QC quiescent center

RAM root apical meristem
RGD3 ROOT GROWTH DEFECTIVE3
RID3 ROOT INITIATION DEFECTIVE3
RIM root-inducing medium
RLE Repressive LEC2 Element
ROS reactive oxygen species
SAM shoot apical meristem
SCR SCARECROW
SERK SOMATIC EMBRYOGENESIS RECEPTOR-LIKE KINASE
SHR SHORTROOT
SIM shoot-inducing medium
STM SHOOTMERISTEMLESS
SWN SWINGER
VAL1 VP1/ABI3-LIKE1
WIND1 WOUND INDUCED DEDIFFERENTIATION 1
WOX5 WUSCHEL-RELATED HOMEOBOX 5
WUS WUSCHEL

1. INTRODUCTION

Plants have evolved various kinds of survival strategies to grow under severe natural conditions. Recovery from damage is an important aspect of survival. In animals, regeneration is mainly required to repair organs or tissues after injury. In contrast, regeneration in plants is not only related to tissue and organ repair, but also to the formation of new plants (Birnbaum & Sanchez Alvarado, 2008; Sugimoto, Gordon, & Meyerowitz, 2011). For example, if branches are cut from a tree trunk, new buds can regenerate (Fig. 1.1A). Many species in the Crassulaceae and Cactaceae families are able to regenerate roots from their detached leaves on the soil (Fig. 1.1B), and subsequently regenerate shoots to form a new plant.

The regenerative abilities of plants have been widely exploited in modern agriculture (Sussex, 2008). Tissue culture, in which plant tissues are cultured on medium supplemented with various plant hormones (Fig. 1.1C and D), exploits the pluripotency and totipotency of plant cells to enable rapid propagation of plant populations. Plant tissue culture has a long history (Gautheret, 1983; Thorpe, 2006, 2007) and this technology is now widely used in agriculture. However, there is still much to learn about the cellular and molecular mechanisms underlying regeneration. In the past few years, molecular genetic approaches have been used to explore plant regeneration, especially that of

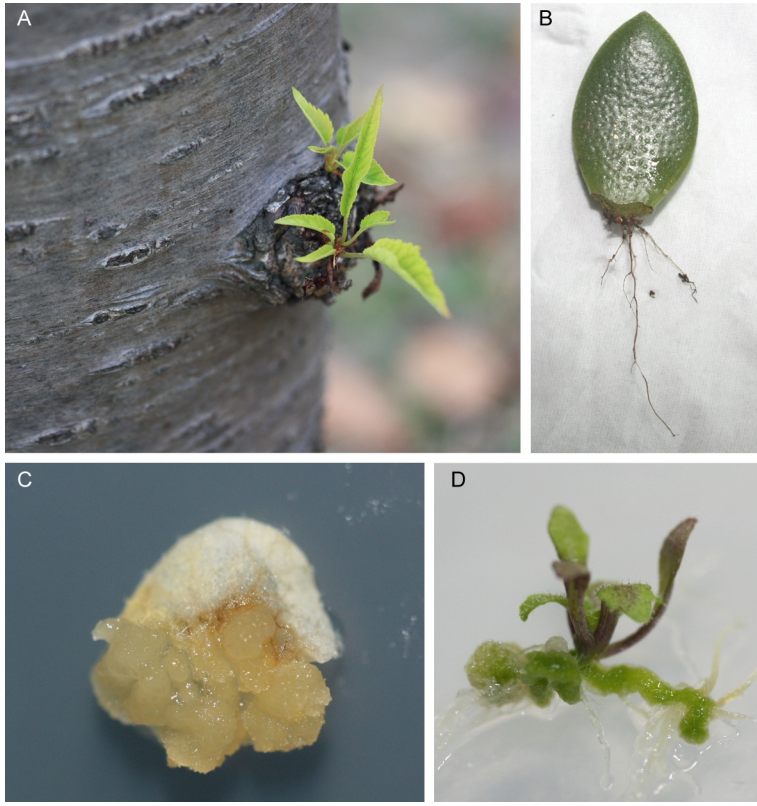

Figure 1.1 Plant regeneration in nature and in tissue culture. (A) Adventitious shoots regenerating from a damaged branch of a tree (*Prunus* × *Yedoensis*). (B) Leaf of a jade plant (*Crassula ovata*) regenerating roots on the soil when detached. (C) Callus regenerated from *Arabidopsis* leaf explants on CIM containing 2.2 μM 2,4-dichlorophenoxyacetic acid (2,4-D) and 0.2 μM kinetin. (D) Shoot regeneration from *Arabidopsis* root explants on SIM containing 12.5 μM 6-(γ,γ-dimethylallylamino) purine (2-IP) and 0.57 μM indole-3-acetic acid (IAA).

model plants, leading to significant progress in the field. In this review, we summarize the recent advances in research on plant regeneration and discuss several new questions related to the plant regeneration process.

2. TYPES OF REGENERATION IN HIGHER PLANTS

Based on differences in cell-fate transition, regeneration of higher plants can be classified into three main categories: tissue regeneration, *de novo* organogenesis, and somatic embryogenesis.

Tissue regeneration (also called tissue repair) refers to the ability to repair wounded sites on the plant body from which tissues have been removed (Fig. 1.2A). This usually occurs after damage to young plant tissues, such as the root or leaf tips. The major function of tissue regeneration is to restore the damaged or removed part; this is comparable to processes in some animals, for example, limb regeneration of amphibians or head regeneration of hydrae and planarians (Sena & Birnbaum, 2010; Sugimoto et al., 2011). In these animals, however, regeneration requires movement of stem cells, whereas plant cells are unable to move. Thus, the mechanisms of regeneration at least partly differ between animals and plants.

Different from tissue regeneration, *de novo* organogenesis results in the development of new organs either from detached organs or from the original plant (Duclercq, Sangwan-Norreel, Catterou, & Sangwan, 2011). "Cuttage" technology, in which new plants are propagated from cuttings, is an example of *de novo* organogenesis for rapid production of new plants in agriculture (Fig. 1.2B). To propagate a new plant from a cutting, a piece of stem is cut and inserted into the soil. Adventitious roots form from the bottom wounds and adventitious shoots form from top or side wounds. These processes represent *de novo* root or shoot organogenesis (also known as *de novo* root or shoot regeneration). In tissue culture, callus formation is also a type of *de novo* organogenesis (Fig. 1.2B). Using *Arabidopsis thaliana* as an example, callus can be induced to form from various detached explants on callus-inducing medium (CIM), which contains a high level of auxin and a low level of cytokinin (Fig. 1.1C and 1.2B). Adventitious roots or shoots can be induced when callus is placed on root-inducing medium (RIM) or shoot-inducing medium (SIM) (Fig. 1.1D and 1.2B), respectively. RIM contains a low level of auxin without cytokinin, while SIM usually contains a high level of cytokinin and a low level of auxin. Since organ formation from callus can be strictly controlled by adding plant hormones to the medium, calli are used for *de novo* organogenesis for different purposes.

Somatic embryogenesis refers to the process in which a single somatic cell dedifferentiates into an embryo cell when cultured under appropriate conditions. The new embryo is able to further develop to form a whole plant (Yang & Zhang, 2010; Zimmerman, 1993) (Fig. 1.2C). The regeneration of a whole plant via somatic embryogenesis reflects the proposed totipotency of plant cells (Verdeil, Alemanno, Niemenak, & Tranbarger, 2007). This process causes a change in cell fate from a somatic cell back into an embryo stem cell and appears to resemble the induction of pluripotent stem (iPS) or embryonic stem (ES) cells from somatic cells in animals (Okita, Ichisaka, & Yamanaka, 2007; Takahashi & Yamanaka, 2006).

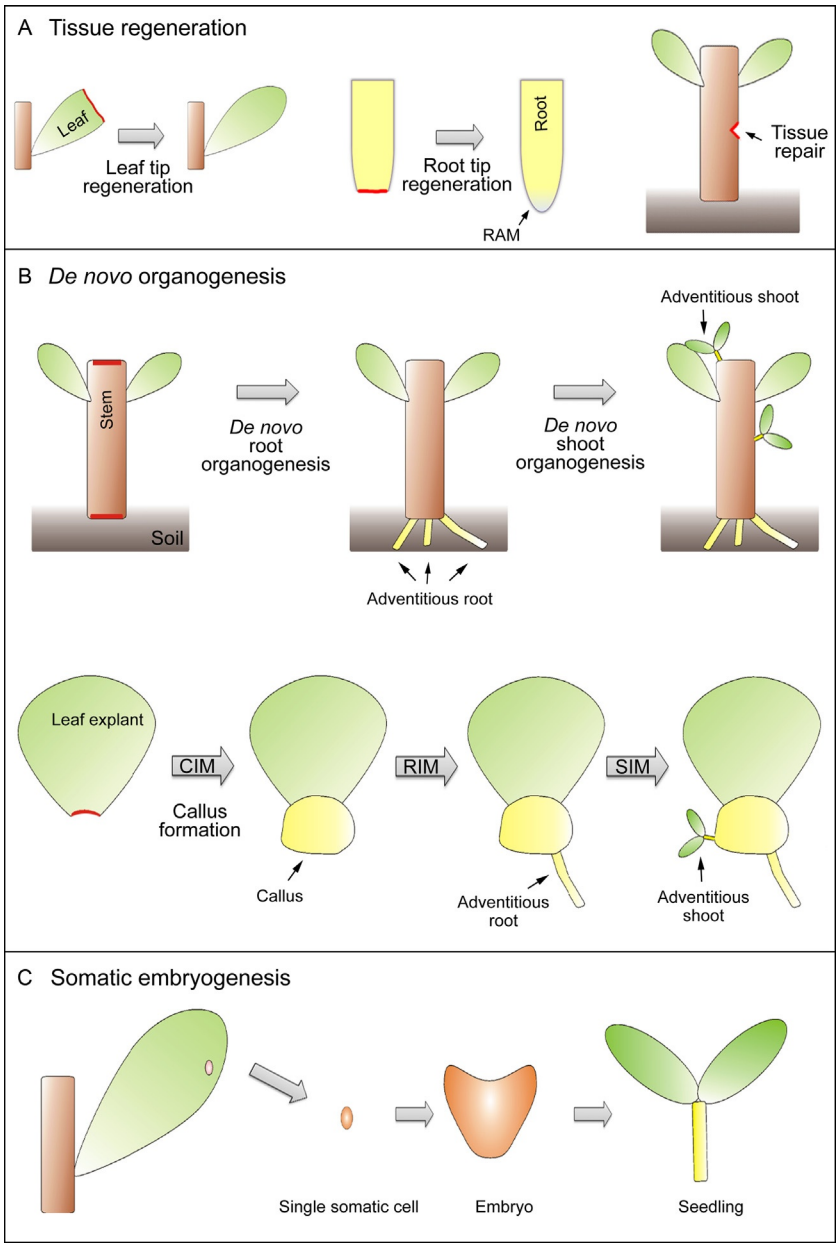

Figure 1.2 Different types of plant regeneration. (A) Tissue regeneration. Excised tips of very young leaves (left) and roots (middle) can re-grow via tissue regeneration. Stem tissues can also be repaired after damage (right). (B) *De novo* organogenesis. Upper panels show *de novo* root and shoot organogeneses during the propagation of plant materials via cuttings. Lower panels show a plant regenerated from leaf explants induced to form callus, adventitious roots, and adventitious shoots on CIM, RIM, and SIM, respectively, in tissue culture. (C) Somatic embryogenesis. A single somatic cell can be induced by auxin to form an embryo, which develops into a whole plant, reflecting the totipotency of plant cells. Red lines in (A) and (B) indicate wounding sites.

3. TISSUE REGENERATION

Damaged tissues usually undergo tissue regeneration. In *Arabidopsis*, studies on tissue regeneration have mainly focused on regeneration of the root meristem and the cellular repair of stem tissues. In both systems, the plant hormone auxin plays a central role in the regeneration processes through regulating key transcription factors.

3.1. Root tip regeneration

The root stem cell niche is at the tip of the root apical meristem (RAM), which comprises a quiescent center (QC) and surrounding stem cells (Aichinger, Kornet, Friedrich, & Laux, 2012; Scheres, 2007). The QC usually has two to four cells that divide very slowly and is marked and controlled by the transcription factor gene *WUSCHEL-RELATED HOMEOBOX 5* (*WOX5*) (Sarkar et al., 2007). An early study on maize showed that, after excision of the root meristem zone, the root quickly regenerated a new QC, leading to subsequent root tip regeneration. These results suggested that the root tip has competence for tissue repair (Feldman, 1976).

Recent studies on root tips of *Arabidopsis* revealed the molecular basis of root tip regeneration (Sena, Wang, Liu, Hofhuis, & Birnbaum, 2009; Xu et al., 2006). After laser-induced ablation of the QC cells, there were dynamic changes in auxin polar transport and in the levels of key transcription factors during the regeneration of the new QC (Xu et al., 2006) (Fig. 1.3A). Before ablation of its cells, the QC was the site of maximum auxin accumulation, as PIN-FORMED (PIN)-mediated polar transport moved auxin from the proximal region of the root to the QC cells. When the QC cells were ablated, auxin instead accumulated in the cells proximally adjacent to the original QC position. The high auxin level led to upregulation of the *PLETHORA* (*PLT*) genes, which promoted the SHORTROOT (SHR) proteins to localize into the nucleus to activate the *SCARECROW* (*SCR*) gene. *PLT*s and *SCR* then acted together to switch the cell fates of the QC-adjacent cells into new QC cells through reorganizing PIN proteins and reactivating *WOX5* expression in the new QC (Xu et al., 2006). Therefore, it appears that the whole process of QC formation is mainly controlled by the auxin level, and a certain high concentration of auxin is the key factor

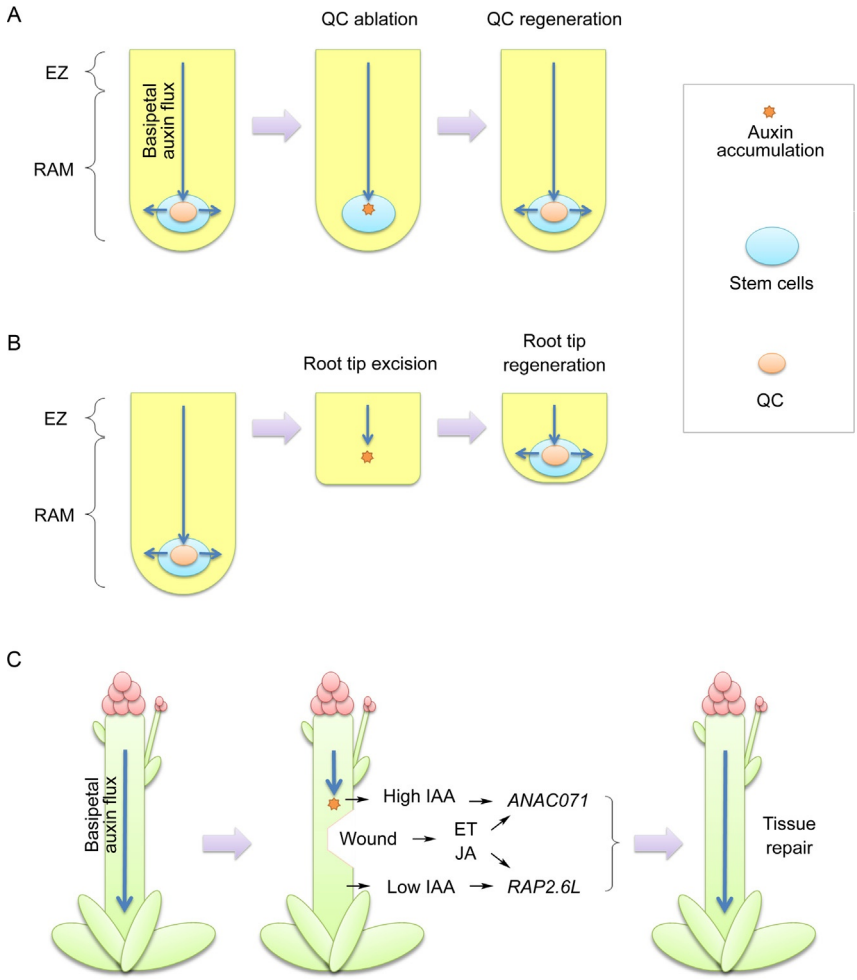

Figure 1.3 Tissue regeneration in *Arabidopsis*. (A) Root tip regeneration after ablation of QC cells (Xu et al., 2006). (B) Root tip regeneration after excision of the root tip (Sena et al., 2009). (C) Tissue repair in the stem (Asahina et al., 2011; Reid & Ross, 2011). Note that the basipetal auxin flux is disrupted by wounding. This results in different auxin levels between upper and lower regions of the wound, triggering the regeneration process to repair the wound. EZ, elongation zone.

determining the QC cell fate. However, it is not yet known whether *PLT*s are the direct target of auxin signaling.

Instead of laser ablation, Sena et al. used a RAM-excision approach to analyze the root regeneration process (Sena et al., 2009) (Fig. 1.3B). The

difference between QC ablation and RAM excision is that only the QC is destroyed by ablation (Xu et al., 2006) whereas the entire stem cell niche is removed by excision (Sena et al., 2009). Therefore, the excision experiment could determine whether a stem cell niche was required to regenerate a new QC. Roots with the RAM removed were able to generate a new QC. Similar to the QC regeneration in the ablation experiment, the newly regenerated QC was controlled by accumulation of high levels of auxin. Treatment with the auxin polar transport-inhibitor naphthylphthalamic acid (NPA) blocked QC regeneration, suggesting that auxin accumulation is pivotal for this process. Since all stem cells were removed in the excision experiment, the newly regenerated QC cells were likely derived from partially differentiated cells. In addition, several stem cell niche-deficient mutants retained the ability of regeneration, indicating that QC regeneration may not require a functional stem cell niche (Sena et al., 2009). However, Sena et al. also found that the QC regeneration capacity was limited to the very distal region of the root tip (the terminal 130 μm region), and the regeneration ability was extremely reduced when a 270-μm portion of the tip was removed (Sena et al., 2009). These findings indicated that fully differentiated cells may not readily change back into QC cells in response to high auxin concentrations, and that cells within the 130 μm of the root tip might retain partial pluripotency.

3.2. Tissue repair in the stem

Recent studies using incision experiments demonstrated how plant tissues repair themselves after wounding (Asahina et al., 2011; Reid & Ross, 2011) (Fig. 1.3C). When the stem is wounded, the basipetal auxin stream is blocked at the wounded position. This results in accumulation of a high level of auxin above the wound and a relatively low auxin level below it. The high auxin level induces expression of the NAC (NAM, ATAF1,2, and CUC2) family transcription factor gene *ANAC071*, while the low auxin level promotes the APETALA2 (AP2)/ERF family transcription factor gene *RAP2.6L*. Both genes are essential for wound healing; however, the molecular roles of the two transcription factors are not yet clear. In addition to the key function of auxin, wound-induced jasmonic acid (JA) and ethylene are also required to regulate *RAP2.6L* (Asahina et al., 2011).

Studies on regeneration in cucumber and tomato revealed that another plant hormone, gibberellin (GA), is involved in wound healing of

hypocotyls (Asahina et al., 2002, 2007). Interestingly, when cotyledons of these plants were removed, the damaged hypocotyls were unable to heal. This phenomenon was caused by the loss of GA derived from the cotyledons, as treatment with GA could rescue the healing defects. These results suggested that GA is also an important hormone for tissue repair. However, the *Arabidopsis* GA-deficient mutant did not show any defects in tissue repair in the stem (Asahina et al., 2011), suggesting that different tissues may require different mechanisms for repair.

4. *De novo* ORGANOGENESIS

De novo organogenesis is a strategy commonly used by plants to survive damage. In nature, *de novo* organogenesis usually occurs to form roots and/or shoots, allowing detached tissues, organs, or wounded plant bodies to develop into new organs or plants. In tissue culture, detached plant tissues or organs on CIM usually first form a pluripotent cell mass, a "callus," from which roots and shoots may grow. It is possible that *de novo* organogenesis in nature also begins with the formation of a tiny pluripotent cell mass, and that the main difference of callus induced in tissue culture is that it is an extremely pronounced form of the "natural" pluripotent cell mass.

4.1. Callus formation

In the 1950s, Skoog and Miller formulated a medium that was suitable for both callus induction and *de novo* organogenesis, and proposed that shoot or root initiation in tissue culture mainly relies on different ratios of auxin and cytokinin in the medium (Skoog & Miller, 1957). After this discovery, callus induction became a widely used method for plant regeneration. Although techniques for callus formation have been used for more than 50 years, the cellular and molecular basis of callus formation was largely unknown until recently. Many plant biology textbooks define callus as a group of undifferentiated totipotent cells, because of its ability to regenerate different types of plant organs. Thus, callus formation from explants was thought to be a dedifferentiation process. However, recent studies in *Arabidopsis* have demonstrated that callus is a mass of root meristem tip cells and that callus induction resembles lateral root formation (Atta et al., 2009; Che, Lall, & Howell, 2007; He, Chen, Huang, & Xu, 2012; Sugimoto, Jiao, & Meyerowitz, 2010) (Fig. 1.4).

The first indication was from phenotypic studies using root and hypocotyl explants to generate callus. Callus formation from these root and hypocotyl explants initiated with divisions of xylem-pole pericycle cells (Atta et al., 2009; Che et al., 2007). Ablation of xylem-pole pericycle cells completely blocked callus formation (Che et al., 2007). The xylem-pole pericycle is the site from which lateral roots form (Benkova & Bielach, 2010; Peret et al., 2009). Therefore, these pericycle cells may serve as pluripotent stem cells that are required for both lateral root initiation and callus formation. The results also suggested that the two biological processes may share similar genetic pathways.

Further important evidence supporting this possibility was reported by Sugimoto et al. (2010). First, a genome-wide gene expression analysis showed that the transcription profile of callus resembled that of the

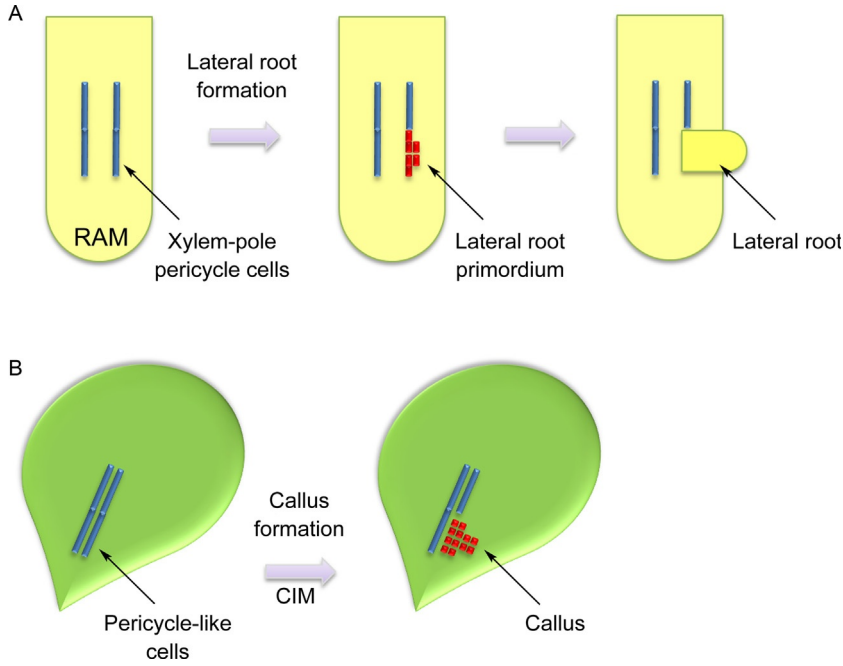

Figure 1.4 Cell-fate transition in callus formation. (A) Model of lateral root formation initiated from xylem-pole pericycle cells. (B) Model of callus formation from a leaf explant. Callus is a cluster of cells resembling root meristem tip cells, which are initiated from pericycle-like cells.

RAM, rather than the shoot apical meristem (SAM) or the embryo, suggesting that callus is a group of root meristem tip cells. Second, expressions of root meristem genes were induced in callus even when it was derived from aerial organs such as petals and cotyledons. Third, callus was shown to be derived from certain cells surrounding the vasculature of aerial organs. These cells express a root xylem-pole pericycle marker J0121 (Laplaze et al., 2005), and therefore, are known as pericycle-like cells. Finally, the lateral-root-initiation deficient mutant *aberrant lateral root formation 4-1* (*alf4-1*) was unable to form callus, suggesting that regulation of callus and lateral root formation at least partially share the same underlying mechanism. All of the results mentioned earlier show that callus formation from aerial organs is not via a dedifferentiation process as previously thought, but forms via a transdifferentiation process. Consequently, callus is no longer considered to be a population of totipotent cells, but rather a pluripotent cell mass (Sugimoto et al., 2010, 2011).

To investigate genetic regulation during callus formation, the gene expression profiles of leaf explants and callus were analyzed (He et al., 2012). The results showed that the leaf-to-callus process comprised at least three stages, each of which was characterized by altered gene expressions. In stage 1, auxin response genes were upregulated; in stage 2, leaf genes were downregulated; and in stage 3, root genes were upregulated (He et al., 2012). These changes in gene expression profiles are consistent with the current hypothesis that callus is a group of root meristem tip cells. Since epigenetic regulation is usually involved in genome-wide regulation of gene expression, He et al. analyzed several mutants corresponding to different histone methylation pathways during callus formation from leaf and cotyledon explants (He et al., 2012). The plant materials in those experiments included the Polycomb group (PcG) mutants *curly leaf-50 swinger-1* (*clf-50 swn-1*) and *embryonic flower2* (*emf2*), both of which are defective in callus formation from leaf explants. CLF, SWN, and EMF2 are components of the Polycomb repressive complex 2 (PRC2) (Chanvivattana et al., 2004; Goodrich et al., 1997; Yang, Chen, & Sung, 1995; Yoshida et al., 2001), which represses gene expression via trimethylation of histone H3 at lysine 27 (H3K27me3) at the target loci (Schubert et al., 2006). In the *clf-50 swn-1* and *emf2* mutants, the levels of H3K27me3 were reduced at the loci of many auxin-response genes and root genes, whereas those at many leaf gene loci were elevated. The proposed role of PcG in the leaf-to-callus transition is to repress leaf genes by depositing H3K27me3 on their loci, because many leaf genes

cannot be silenced during callus formation from leaf explants in the PcG mutants (He et al., 2012). However, it is not clear whether demethylation of H3K27me3 also plays a role in derepressing the auxin-response genes and root genes. It is possible that in addition to the PcG pathway, many other epigenetic pathways are also involved in gene regulation during callus formation. Furthermore, in future research, it will be important to determine how epigenetic pathways specifically regulate their targets.

An important finding about gene regulation during callus formation was that several *LATERAL ORGAN BOUNDARIES DOMAIN* (*LBD*) family genes play roles in the process, providing a molecular link between auxin signaling and the establishment of root-cell fate (Fan, Xu, Xu, & Hu, 2012). *LBD16*, *17*, *18*, and *29* are key genes in lateral root formation (Berckmans et al., 2011; Feng, Sun, Wang, Liu, & Zhu, 2012; Feng, Zhu, Du, & Cui, 2012; Lee, Kim, Kim, Lee, & Kim, 2013; Okushima, Fukaki, Onoda, Theologis, & Tasaka, 2007), and recent research showed that they are also critical in callus formation (Fan et al., 2012). Overexpression of *LBD* genes resulted in spontaneous callus formation without an increase in exogenous auxin levels, suggesting that *LBD*s act downstream of auxin signaling (Fan et al., 2012). Previous studies showed that these *LBD* genes are the direct targets of auxin signaling, and that LBD proteins control cell proliferation and cell wall loosening during lateral root initiation (Berckmans et al., 2011; Lee et al., 2013; Okushima et al., 2007). Therefore, it is possible that during callus formation, the *LBD* genes in explant tissues respond to high auxin levels in the CIM and initiate cell division to form callus (Fan et al., 2012).

Another interesting discovery was that in rice, genome-wide DNA methylation patterns always change during callus formation (Stroud et al., 2013). These changes are inherited across generations after the callus regenerates into whole plants. Therefore, it seems that the plants regenerated from callus may harbor epigenetic information different from that of the original (preculture) plant (Stroud et al., 2013). It is still unknown whether other epigenetic regulation mechanisms such as histone modifications and chromatin remodeling coordinate with DNA methylation during rice callus formation.

The process of callus formation from explants is correlated with cell-fate transition, accompanied by large-scale changes in gene expression (Che, Lall, Nettleton, & Howell, 2006; He et al., 2012; Pischke, Huttlin, Hegeman, & Sussman, 2006; Sugimoto et al., 2010; Xu et al., 2012). Although it has been established that the cell-fate transition during callus

formation resembles that during lateral root formation and several key factors in this process have been identified, the mechanisms by which these processes are precisely and gradually programmed still need to be investigated in the future.

4.2. *De novo* root organogenesis

De novo root organogenesis occurs commonly in many plant species when detached organs meet suitable conditions, for example, when organs fall on wet and nutrient-rich soil. Some plants, such as those in the Crassulaceae and Cactaceae families, use *de novo* root organogenesis to proliferate their populations (Fig. 1.1B). However, the molecular mechanism directing this rooting process is unclear. In recent studies on *Arabidopsis*, various systems have been established to study *de novo* root organogenesis from excised leaves and hypocotyls (Correa Lda, Troleis, Mastroberti, Mariath, & Fett-Neto, 2012). Interestingly, adventitious roots originate from the vascular procambium or cambium in *Arabidopsis*, suggesting that the procambium or cambium cells may serve as potential pluripotent stem cells for regeneration (Ahkami et al., 2009; Correa Lda et al., 2012; Greenwood, Cui, & Xu, 2001). In addition, inhibition of auxin polar transport completely blocked the rooting process of leaf explants, suggesting that auxin biology must be involved in *de novo* root organogenesis (our unpublished data). It is possible that *de novo* root organogenesis is similar to lateral or adventitious root formation from hypocotyls. However, *de novo* root organogenesis from detached organs is triggered by the wound signal, which is absent during lateral or adventitious root formation from hypocotyls. The molecular nature of the wound signal has not yet been defined. Also, it is still unknown how the wounding signal controls auxin biology and cell-fate transition in the procambium or cambium. Understanding these processes is crucial to unraveling the molecular mechanisms of *de novo* root organogenesis.

4.3. *De novo* shoot organogenesis

In nature, *de novo* shoot organogenesis usually occurs on wounded plant stems (see example in Fig. 1.1A). In tissue culture, callus can also be induced to undergo shoot organogenesis (see example in Fig. 1.1D). Histological studies using different explants showed that two types of cells give rise to regenerated shoots. In *Arabidopsis* root explants, adventitious shoots emerged from the xylem-pole pericycle cells on SIM (Atta et al., 2009; Che et al., 2007). In peach palm shoot apex explants, adventitious shoots were initiated from the preprocambium (de Almeida, de Almeida,

Mendes Graner, Ebling Brondani, & Fiori de Abreu-Tarazi, 2012). These results suggest that xylem-pole pericycle cells in roots and preprocambium cells in aerial organs contain pluripotent stem cells for *de novo* shoot regeneration. Their origin in the xylem-pole pericycle and the preprocambium might be important for the newly formed shoots to capture water and nutrients from the explants via the vascular system.

In fact, xylem-pole pericycle, preprocambium, procambium, and cambium cells all have stem cell features. Procambium and cambium are pluripotent vascular meristem in primary development and secondary development of vascular tissues, respectively (Elo, Immanen, Nieminen, & Helariutta, 2009). Preprocambium is the progenitor of procambium (Elo et al., 2009). As well, in terms of cell lineage, the cambium in roots develops partly from xylem-pole pericycle cells (Rost, Barbour, Stocking, & Murphy, 1997). It is possible that only stem cells are competent to regenerate roots or shoots in *de novo* organogenesis. Since *de novo* shoot organogenesis is initiated from the preprocambium or xylem-pole pericycle cells, it seems that this regeneration process shares some similarities with that of lateral root formation, rather than lateral shoot formation. Lateral shoots originate from external cells on the adaxial side of leaf axils, while adventitious shoots formed via *de novo* shoot organogenesis initiate from internal xylem-pole pericycle or preprocambium cells.

In tissue culture, callus can be easily induced to undergo *de novo* shoot organogenesis on SIM containing a high level of cytokinin, suggesting that cytokinin is the key hormone for shoot induction, just like auxin is the key hormone for root induction (Duclercq, Sangwan-Norreel, et al., 2011; Skoog & Miller, 1957). This is consistent with several features of SAM and RAM development in *Arabidopsis*. In the RAM, the highest level of auxin is in the QC region, suggesting a role of auxin in controlling or maintaining the QC (Sabatini et al., 1999). Similarly, in the SAM, cytokinin has an essential role to control the organizing center (OC) (Bartrina, Otto, Strnad, Werner, & Schmulling, 2011; Gordon, Chickarmane, Ohno, & Meyerowitz, 2009; Leibfried et al., 2005; Rupp, Frank, Werner, Strnad, & Schmulling, 1999; Sablowski, 2009; Yanai et al., 2005; Zhao et al., 2010). On the other hand, auxin could be an assistant factor in shoot regeneration, just like cytokinins can be an assistant factor in rooting (Cheng et al., 2013; Skoog & Miller, 1957).

Several *Arabidopsis* mutants that are defective in *de novo* shoot organogenesis are also defective in their cytokinin or auxin pathways (Che, Gingerich, Lall, & Howell, 2002; Che, Lall, & Howell, 2008; Che et al., 2006;

Hwang & Sheen, 2001; Inoue et al., 2001; Pernisova et al., 2009; Sakai et al., 2001). However, the mechanism by which hormone signaling controls the shooting process is unclear. One possibility is that these hormones upregulate key SAM-related genes, such as *CUP-SHAPED COTYLEDON2* (*CUC2*), *WUSCHEL* (*WUS*), and *SHOOTMERISTEMLESS* (*STM*). A detailed analysis of gene expression using real-time fluorescence techniques revealed expression patterns of several key genes during shooting from callus on SIM (Gordon et al., 2007). Correct distributions of *WUS* and *CUC2* appeared to be essential for the shoot progenitor cells, because *CUC2* was expressed in the shoot progenitor that was surrounded by cells expressing *WUS*. The proliferation of the shoot progenitor led to the radial patterning of the newly formed shoot promeristem, accompanied by *STM* expression in the region surrounding it. At this stage, PIN1 was also distributed in the surface of the shoot promeristem, suggesting that polar auxin transport is also required for the organization of the SAM (Gordon et al., 2007).

Upregulations of *WUS* and *STM* are important for shooting on SIM (Chatfield et al., 2013; Cheng, Zhu, Gao, & Zhang, 2010; Gordon et al., 2007; Tamaki et al., 2009). Interestingly, upregulation of *WUS* requires epigenetic regulation, as mutations in the DNA methyltransferase gene *METHYLTRANSFERASE1* (*MET1*), the histone H3K9 methyltransferase gene *KRYPTONITE* (*KYP*), the H3K4 demethylase JMJ14, and the histone acetyltransferase *HAC1* all resulted in altered *WUS* expression and affected the shooting rate (Li et al., 2011). It was proposed that DNA methylation and H3K9me2 function to repress *WUS* expression in callus. Thus, removal of these two epigenetic modifications resulted in *WUS* derepression, which facilitated shooting (Li et al., 2011).

Several other genes are also involved in regulating *de novo* shoot organogenesis in tissue culture. *ENHANCER OF SHOOT REGENERATION1* (*ESR1*) and *ESR2* both encode AP2/ERF domain transcription factors that control shooting (Banno, Ikeda, Niu, & Chua, 2001; Matsuo, Makino, & Banno, 2011). *ESR2* promotes shooting via upregulation of *CUC1* expression (Ikeda, Banno, Niu, Howell, & Chua, 2006). The *hoc* mutant, which is defective in the class III HD-ZIP family gene *ATHB15/CORONA/INCURVATA4*, overproduces cytokinin, facilitating shoot regeneration from root explants (Catterou et al., 2002; Duclercq, Assoumou Ndong, Guerineau, Sangwan, & Catterou, 2011). In addition, the temperature-sensitive mutants *srd*s, *root initiation defective3* (*rid3*) and *root growth defective3* (*rgd3*) were shown to be defective in *de novo*

shoot organogenesis (Ohtani, Demura, & Sugiyama, 2008; Ohtani & Sugiyama, 2005; Ozawa, Yasutani, Fukuda, Komamine, & Sugiyama, 1998; Tamaki et al., 2009; Yasutani, Ozawa, Nishida, Sugiyama, & Komamine, 1994). Although genes that regulate *de novo* shoot organogenesis have been identified, the network from hormones to key genes, including epigenetic regulations, has not yet been established. Evidence for direct molecular interactions is also lacking.

5. SOMATIC EMBRYOGENESIS

In theory, somatic embryogenesis is a typical dedifferentiation process that returns a differentiated somatic cell back to a totipotent embryo stem cell state. The early experiments in which carrot cells were induced to undergo somatic embryogenesis (Steward, Mapbs, & Mears, 1958) provided strong evidence for the plant totipotency theory in early plant cell biology studies. Recent studies suggest that somatic embryogenesis is a complex process involving hormone actions, transcription factors, and epigenetic regulations (Yang & Zhang, 2010).

5.1. Altered hormone balance in somatic embryogenesis

The balance between two plant hormones, GA and abscisic acid (ABA), is pivotal in controlling cells in the embryo or the postembryo state (de Castro & Hilhorst, 2006; Hays, Mandel, & Pharis, 2001; Hu et al., 2008; Ogawa et al., 2003; Phillips et al., 1997; Vahdati, Bayat, Ebrahimzadeh, Jariteh, & Mirmasoumi, 2008; White, Proebsting, Hedden, & Rivin, 2000; Yamaguchi, Kamiya, & Nambara, 2007) (Fig. 1.5). Embryo cells usually have a low ratio of GA to ABA, while this ratio is higher in somatic cells (Braybrook & Harada, 2008). These findings suggested that the cell-fate transition from somatic cells to embryo cells requires an increased ABA level and a decreased GA level.

Auxin, especially the synthetic auxin 2,4-D, is commonly used in culture medium to trigger somatic embryogenesis (Bai, Su, Yuan, & Zhang, 2013; Elhiti et al., 2013; Gaj, 2004; Ikeda-Iwai, Satoh, & Kamada, 2002; Jimenez, 2005; Luo & Koop, 1997; Michalczuk, Ribnicky, Cooke, & Cohen, 1992; Raghavan, 2004; Su & Zhang, 2009; Su et al., 2009). It was proposed that auxin might induce and initiate somatic embryogenesis, whereas changes in the GA/ABA ratio could provide a suitable environment for cells to become competent to form a somatic embryo (Braybrook & Harada, 2008). In addition, ethylene was also shown to

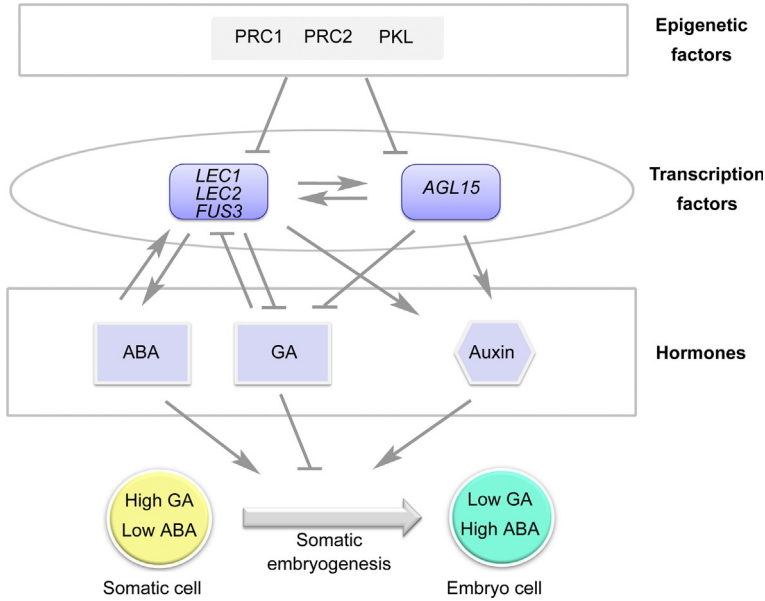

Figure 1.5 Regulation of somatic embryogenesis. Epigenetic and transcription factors and plant hormones are involved in somatic embryogenesis. Embryo and somatic cells are characterized by different GA/ABA ratios, and auxin triggers the induction of somatic embryogenesis. Plant hormones and transcription factors form a network with cross talk, and epigenetic pathways involving PcG and PKL repress expression of embryo genes to prevent somatic cells from bearing embryo traits.

be involved in the somatic cell-to-embryonic cell transition (Bai et al., 2013; Piyatrakul et al., 2012; Zheng, Zheng, & Perry, 2013).

5.2. Key transcription factors in somatic embryogenesis

Two types of transcription factors play essential roles in somatic embryo formation in *Arabidopsis*: *LEAFY COTYLEDON* (*LEC*) genes and the *AGAMOUS-LIKE15* (*AGL15*) gene (Braybrook & Harada, 2008; Harding, Tang, Nichols, Fernandez, & Perry, 2003) (Fig. 1.5). The *LEC* genes include *LEC1*, which encodes the HAP3 subunit of the CCAAT-binding transcription factor (Kwong et al., 2003; Lotan et al., 1998), and *LEC2* and *FUSCA3* (*FUS3*), which encode B3-domain proteins (Luerssen, Kirik, Herrmann, & Misera, 1998; Stone et al., 2001). *AGL15* encodes a MADS-box transcription factor (Harding et al., 2003; Heck, Perry, Nichols, & Fernandez, 1995; Rounsley, Ditta, & Yanofsky, 1995).

These genes are expressed exclusively in embryos. Ectopic overexpression of each of these genes resulted in embryonic traits in somatic tissues or the formation of somatic embryos (Harding et al., 2003; Lotan et al., 1998; Stone et al., 2001). These properties are reminiscent of those of Yamanaka factors, which induce adult cells to become iPS cells or ES cells in animals (Okita et al., 2007; Takahashi & Yamanaka, 2006).

LEC and *AGL15* genes can promote expression of each other and determine embryo cell fate by cross talk among hormone pathways (Braybrook & Harada, 2008; Braybrook et al., 2006; Zheng, Ren, Wang, Stromberg, & Perry, 2009). Molecular analyses suggested that *AGL15* inhibits the GA pathway via promoting *GA2ox6* expression and repressing *GA3ox2* expression (Wang, Caruso, Downie, & Perry, 2004; Zheng et al., 2009), and upregulates the auxin signaling gene *IAA30* (Zheng et al., 2009). *FUS3* inhibits the GA pathway via repressing the GA biosynthesis genes *GA3ox1* and *GA3ox2* (Curaba et al., 2004; Gazzarrini, Tsuchiya, Lumba, Okamoto, & McCourt, 2004). In addition, the *FUS3* pathway and the ABA action pathway positively regulate each other, although the molecular mechanism remains unclear (Gazzarrini et al., 2004; Kagaya, Okuda, et al., 2005; Kagaya, Toyoshima, et al., 2005). *LEC2* promotes the auxin pathway by upregulating the auxin biosynthesis genes *YUC2* and *YUC4* and the *IAA30* gene (Braybrook et al., 2006; Stone et al., 2008). All of the transcription factors described earlier make up a genetic network that promotes the ABA and auxin pathways but inhibits the GA pathway. On the other hand, their expressions also respond to changes in the levels of these hormones. Therefore, somatic embryogenesis requires this highly complex network.

Several other transcription factors also play roles in regulating somatic embryogenesis. *PGA37/MYB118* and *MYB115* positively regulate *LEC1* expression (Wang et al., 2009). Overexpression of *BABY BOOM* (*BBM*) and *EMBRYOMAKER* (*EMK*), both of which encode AP2-domain transcription factors, caused somatic embryogenesis (Boutilier et al., 2002; Tsuwamoto, Yokoi, & Takahata, 2010). *ABI3*, which encodes a B3 domain transcription factor, was positively regulated by *LEC*s and *AGL15* (Giraudat et al., 1992; Kagaya, Toyoshima, et al., 2005; Zheng et al., 2009). A double mutation of the B3 domain genes *VP1/ABI3-LIKE1* (*VAL1*) and *VAL2* resulted in somatic embryogenesis with ectopic expressions of *LEC1*, *ABI3*, and *FUS3*, suggesting that *VAL1* and *VAL2* are negative regulators of somatic embryogenesis (Suzuki, Wang, & McCarty, 2007). *WUS* and other SAM-related genes were also shown to play roles in promoting

somatic embryogenesis (Bouchabke-Coussa et al., 2013; Elhiti, Tahir, Gulden, Khamiss, & Stasolla, 2010; Mordhorst, Hartog, El Tamer, Laux, & de Vries, 2002; Su et al., 2009; Zuo, Niu, Frugis, & Chua, 2002). In addition, SOMATIC EMBRYOGENESIS RECEPTOR-LIKE KINASEs (SERKs) might be involved in a signaling pathway in somatic embryogenesis via forming a protein complex with AGL15 (Hecht et al., 2001; Karlova et al., 2006).

5.3. Epigenetic regulation of somatic embryogenesis

Epigenetic pathways are critical to retain somatic features during post-embryonic development of *Arabidopsis* via repressing embryo-specific genes. There are two major epigenetic pathways that prevent ectopic expression of embryo genes in somatic cells: the PcG and PICKLE (PKL) pathways (Fig. 1.5). Mutations in these pathways can result in somatic embryogenesis.

The PcG pathway is important to retain somatic cell identity. Two PcG complexes, PRC1 and PRC2, have been identified from *Arabidopsis*. Genome-wide reprogramming of PRC2-mediated H3K27me3 is not only involved in leaf-to-callus formation as mentioned earlier (He et al., 2012), but also in repressing embryonic genes during the embryo-to-seedling phase transition (Bouyer et al., 2011). Mutations in PRC2 components resulted in an incomplete transition from embryo to seedling, and the aberrant mutant seedling exhibited disorganized cell divisions and callus-like tissues with embryo traits (Bouyer et al., 2011; Chanvivattana et al., 2004). *LEC1*, *LEC2*, and *FUS3* were ectopically expressed in the PRC2 component double mutant *clf swn* (Makarevich et al., 2006). Genome-wide analysis of H3K27me3 distribution suggested that these embryo-specific genes are highly trimethylated in somatic cells (Zhang, Clarenz, et al., 2007). Recently, a *cis* element, named *Repressive LEC2 Element* (*RLE*), was identified in the *LEC2* promoter; the function of *RLE* is to recruit PRC2 for trimethylation at the *LEC2* locus (Berger, Dubreucq, Roudier, Dubos, & Lepiniec, 2011).

PRC1 is currently thought to contain four major proteins in *Arabidopsis*: LIKE HETEROCHROMATIN PROTEIN1 (LHP1, also called TERMINAL FLOWER2) (Gaudin et al., 2001; Kotake, Takada, Nakahigashi, Ohto, & Goto, 2003), AtRING1a/b (Xu & Shen, 2008), AtBMI1a/b (Bratzel, Lopez-Torrejon, Koch, Del Pozo, & Calonje, 2010; Chen,

Molitor, Liu, & Shen, 2010), and EMF1 (Aubert et al., 2001; Bratzel et al., 2010). LHP1 serves as a reader to recognize H3K27me3 (Turck et al., 2007; Zhang, Germann, et al., 2007), and the RING-domain proteins AtRING1a/b and AtBMI1a/b are responsible for H2A ubiquitination (H2Aub) after PRC1 binds to its targets (Bratzel et al., 2010). Mutations in AtRING1a/b and AtBMI1a/b resulted in strongly ectopic embryo traits in somatic tissues and ectopic expression of *LEC*s, *FUS3*, *AGL15*, and *ABI3* (Bratzel et al., 2010; Chen et al., 2010). A recent study showed that VAL proteins might recruit PRC1-mediated H2Aub to initiate repression of the embryo genes, and the repression state is then maintained by PRC2-mediated H3K27me3 (Yang et al., 2013). These results suggested that PRC1 and PRC2 may function together to control the same embryonic genes.

PKL is another epigenetic factor that plays a critical role in preventing somatic cells from bearing embryonic traits. *PKL* encodes a CHD-type ATP-dependent chromatin remodeling factor, and loss of function in *PKL* was first identified as a GA-deficient mutant (Ogas, Cheng, Sung, & Somerville, 1997; Ogas, Kaufmann, Henderson, & Somerville, 1999). Chromatin remodeling factors function to regulate gene expression via rearranging nucleosome positions (Clapier & Cairns, 2009). Mutations in *PKL* resulted in ectopic expression of *LEC1*, *LEC2*, and *FUS3* in the root tip, and therefore, caused the formation of a pickle-like root phenotype (Dean Rider et al., 2003). A recent study showed that *PICKLE-RELATED2* (*PKR2*), a *PKL* homolog in *Arabidopsis*, plays redundant roles with *PKL* in repressing embryonic traits in the root. A mutation in *PKR2* in the *pkl* mutant background enhanced the pickle-like root phenotype (Aichinger et al., 2009). However, the molecular mechanism by which PKL represses *LEC* genes is still arguable. It was proposed that PKL might repress these embryonic genes via promoting H3K27me3 (Zhang, Bishop, Ringenberg, Muir, & Ogas, 2012; Zhang et al., 2008), whereas other data suggested that PKL promotes expression of PRC2 genes to indirectly repress *LEC1* and *FUS3* (Aichinger et al., 2009). In future research, it will be important to clarify the molecular role of PKL in somatic embryogenesis. A genome-wide ChIP-seq analysis to determine the localization of PKL may be a useful strategy toward this goal. On the other hand, PKL also negatively regulates cytokinin responses in callus formation (Furuta et al., 2011), suggesting its multiple roles in plant regeneration.

6. WOUND SIGNAL IN PLANT REGENERATION

All three types of plant regeneration are triggered by wounding, indicating that this is the original signal(s). This wound signal causes changes in hormone biology, leading to changes in gene expression. However, the molecular nature of the wound signal remains unclear. It is possible that it is a complex mixture of different chemicals and that the signal varies among different tissues/organs or under different wounding conditions. This is because wounding can induce different types of regeneration when different kinds or portions of tissues/organs are damaged or detached. Studies on wounding have suggested several possible candidates, such as plasma transmembrane potential, Ca^{2+}, reactive oxygen species (ROS), plant hormones, and changes in various metabolic processes (Leon, Rojo, & Sanchez-Serrano, 2001; Maffei, Mithofer, & Boland, 2007). The wound signal triggers not only regeneration but also the defense response; therefore, its signal transduction pathway could be very complex.

Recent studies have focused on the *Arabidopsis* wound-responsive gene, *WOUND INDUCED DEDIFFERENTIATION 1* (*WIND1*) (Iwase et al., 2011; Iwase, Ohme-Takagi, & Sugimoto, 2011). *WIND1* encodes an AP2/ERF domain transcription factor, and expression of *WIND1* is induced at the wounded region. Ectopic expression of *WIND1* promotes cell proliferation and callus formation from somatic cells. *WIND1* appeared to function in regulating plant regeneration via the cytokinin pathway (Iwase, Mitsuda, et al., 2011; Iwase, Ohme-Takagi, & Sugimoto, 2011). However, it is still not clear how wounding induces *WIND1*. Detailed analysis of the *WIND1* promoter may reveal aspects of its upstream signaling pathway.

7. REGENERATION IN MOSS

The moss *Physcomitrella patens* is an ideal model plant for studying plant regeneration because many different types of tissues or cells are easily triggered to undergo cell-fate transition and regeneration. For example, a single protoplast from moss can regenerate into the protonema apical cell. This phenomenon was proposed to be the result of a reprogramming process from differentiated cells into stem cells with expressional changes of thousands of genes (Xiao, Zhang, Yang, Zhu, & He, 2012).

Another example of moss regeneration is the *de novo* regeneration of chloronema apical cells from excised gametophyte leaf cells (Ishikawa et al., 2011). Regeneration occurred rapidly without addition of any exogenous hormones; thus, the cell-fate transition probably depends on its own endogenous hormones. Ishikawa et al. used different cell proliferation inhibitors to test the relationship between cell division and cell-fate transition during moss regeneration. An interesting discovery was that expression of the *cyclin-dependent kinase A* (*CDKA*) gene was induced prior to cell-fate transition and cell division. Furthermore, addition of the DNA synthesis inhibitor aphidicolin to stop the cell cycle neither prevented the cell-fate transition from leaf cell to chloronema apical cell, nor blocked the induction of protonema-specific genes. This suggested that cell-fate transition occurs before, and is independent of, cell division during moss regeneration. However, addition of the CDKA-inhibitor roscovitine to the medium blocked both cell-fate transition and cell division, suggesting that CDKA has dual roles in controlling cell-fate transition and cell division during regeneration (Ishikawa et al., 2011). It is not known whether these separate cell-fate transition and cell division processes that occur during moss regeneration commonly occur in other types of plant regeneration. It is possible that the initiation of cell-fate transition is first triggered by plant hormones and key transcription factor genes, and then cell division proceeds to form new tissues and organs.

8. CONCLUDING REMARKS AND PERSPECTIVES

The underlying theme of regeneration is cell-fate transition (cell-fate reprogramming), and different types of regeneration involve different kinds of cell-fate transition. For example, root QC regeneration involves cell-fate transition from the root meristem or partially differentiated cell into the QC cell; callus regeneration involves cell-fate transition from pericycle-like cells into root meristem cells; and somatic embryogenesis involves cell-fate transition from somatic cells into embryo cells. Not all of these fate transitions represent a dedifferentiation process; in fact, callus formation from aerial organs is a transdifferentiation process. Cell-fate transitions are usually accompanied by the actions of plant hormones, which trigger transcription factors and epigenetic factors to reprogram the genome-wide gene expression profile. Transcription factors and epigenetic factors also regulate hormone actions via effects on their biosyntheses and signaling, forming feedback networks. Therefore, plant regeneration could be a good platform

to study molecular mechanisms of cell-fate transition in plants. It is interesting that some regeneration processes share similar regulatory mechanisms with those of developmental processes. For example, callus formation resembles lateral root formation. Therefore, studies on regeneration may also provide clues about plant developmental biology.

Although recent studies on plant regeneration have greatly increased our understanding of the plasticity of plant cells, many questions remain unanswered. What is the wound signal that triggers the regeneration process? Which gene(s) is the direct target of plant hormones for cell-fate transition? How do transcription and epigenetic factors corporately act to regulate their downstream events? In addition, Sugimoto et al. provided important findings about callus formation from aerial organs, but the callus initiators, the so-called pericycle-like cells, have not been identified. All these points should be explored in future research.

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REFERENCES

Ahkami, A. H., Lischewski, S., Haensch, K. T., Porfirova, S., Hofmann, J., Rolletschek, H., et al. (2009). Molecular physiology of adventitious root formation in *Petunia hybrida* cuttings: Involvement of wound response and primary metabolism. *The New Phytologist*, *181*, 613–625.

Aichinger, E., Kornet, N., Friedrich, T., & Laux, T. (2012). Plant stem cell niches. *Annual Review of Plant Biology*, *63*, 615–636.

Aichinger, E., Villar, C. B., Farrona, S., Reyes, J. C., Hennig, L., & Kohler, C. (2009). CHD3 proteins and polycomb group proteins antagonistically determine cell identity in Arabidopsis. *PLoS Genetics*, *5*, e1000605.

Asahina, M., Azuma, K., Pitaksaringkarn, W., Yamazaki, T., Mitsuda, N., Ohme-Takagi, M., et al. (2011). Spatially selective hormonal control of RAP2.6L and ANAC071 transcription factors involved in tissue reunion in *Arabidopsis*. *Proceedings of the National Academy of Sciences of the United States of America*, *108*, 16128–16132.

Asahina, M., Iwai, H., Kikuchi, A., Yamaguchi, S., Kamiya, Y., Kamada, H., et al. (2002). Gibberellin produced in the cotyledon is required for cell division during tissue reunion in the cortex of cut cucumber and tomato hypocotyls. *Plant Physiology*, *129*, 201–210.

Asahina, M., Yamauchi, Y., Hanada, A., Kamiya, Y., Kamada, H., Satoh, S., et al. (2007). Effects of the removal of cotyledons on endogenous gibberellin levels in hypocotyls of young cucumber and tomato seedlings. *Plant Biotechnol*, *24*, 99–106.

Atta, R., Laurens, L., Boucheron-Dubuisson, E., Guivarc'h, A., Carnero, E., Giraudat-Pautot, V., et al. (2009). Pluripotency of Arabidopsis xylem pericycle underlies shoot regeneration from root and hypocotyl explants grown *in vitro*. *The Plant Journal*, *57*, 626–644.

Aubert, D., Chen, L., Moon, Y. H., Martin, D., Castle, L. A., Yang, C. H., et al. (2001). EMF1, a novel protein involved in the control of shoot architecture and flowering in Arabidopsis. *Plant Cell*, *13*, 1865–1875.

Bai, B., Su, Y. H., Yuan, J., & Zhang, X. S. (2013). Induction of somatic embryos in *Arabidopsis* requires local *YUCCA* expression mediated by the down-regulation of ethylene biosynthesis. *Molecular Plant*, *6*, 1247–1260.

Banno, H., Ikeda, Y., Niu, Q. W., & Chua, N. H. (2001). Overexpression of Arabidopsis *ESR1* induces initiation of shoot regeneration. *Plant Cell*, *13*, 2609–2618.

Bartrina, I., Otto, E., Strnad, M., Werner, T., & Schmulling, T. (2011). Cytokinin regulates the activity of reproductive meristems, flower organ size, ovule formation, and thus seed yield in *Arabidopsis thaliana*. *Plant Cell*, *23*, 69–80.

Benkova, E., & Bielach, A. (2010). Lateral root organogenesis—From cell to organ. *Current Opinion in Plant Biology*, *13*, 677–683.

Berckmans, B., Vassileva, V., Schmid, S. P., Maes, S., Parizot, B., Naramoto, S., et al. (2011). Auxin-dependent cell cycle reactivation through transcriptional regulation of *Arabidopsis E2Fa* by lateral organ boundary proteins. *Plant Cell*, *23*, 3671–3683.

Berger, N., Dubreucq, B., Roudier, F., Dubos, C., & Lepiniec, L. (2011). Transcriptional regulation of *Arabidopsis LEAFY COTYLEDON2* involves *RLE*, a *cis*-element that regulates trimethylation of histone H3 at lysine-27. *Plant Cell*, *23*, 4065–4078.

Birnbaum, K. D., & Sanchez Alvarado, A. (2008). Slicing across kingdoms: Regeneration in plants and animals. *Cell*, *132*, 697–710.

Bouchabke-Coussa, O., Obellianne, M., Linderme, D., Montes, E., Maia-Grondard, A., Vilaine, F., et al. (2013). Wuschel overexpression promotes somatic embryogenesis and induces organogenesis in cotton (*Gossypium hirsutum* L.) tissues cultured in vitro. *Plant Cell Reports*, *32*, 675–686.

Boutilier, K., Offringa, R., Sharma, V. K., Kieft, H., Ouellet, T., Zhang, L., et al. (2002). Ectopic expression of BABY BOOM triggers a conversion from vegetative to embryonic growth. *Plant Cell*, *14*, 1737–1749.

Bouyer, D., Roudier, F., Heese, M., Andersen, E. D., Gey, D., Nowack, M. K., et al. (2011). Polycomb repressive complex 2 controls the embryo-to-seedling phase transition. *PLoS Genetics*, *7*, e1002014.

Bratzel, F., Lopez-Torrejon, G., Koch, M., Del Pozo, J. C., & Calonje, M. (2010). Keeping cell identity in *arabidopsis* requires PRC1 RING-finger homologs that catalyze H2A monoubiquitination. *Current Biology*, *20*, 1853–1859.

Braybrook, S. A., & Harada, J. J. (2008). LECs go crazy in embryo development. *Trends in Plant Science*, *13*, 624–630.

Braybrook, S. A., Stone, S. L., Park, S., Bui, A. Q., Le, B. H., Fischer, R. L., et al. (2006). Genes directly regulated by LEAFY COTYLEDON2 provide insight into the control of embryo maturation and somatic embryogenesis. *Proceedings of the National Academy of Sciences of the United States of America*, *103*, 3468–3473.

Catterou, M., Dubois, F., Smets, R., Vaniet, S., Kichey, T., Van Onckelen, H., et al. (2002). *hoc*: An *Arabidopsis* mutant overproducing cytokinins and expressing high *in vitro* organogenic capacity. *The Plant Journal*, *30*, 273–287.

Chanvivattana, Y., Bishopp, A., Schubert, D., Stock, C., Moon, Y. H., Sung, Z. R., et al. (2004). Interaction of Polycomb-group proteins controlling flowering in *Arabidopsis*. *Development*, *131*, 5263–5276.

Chatfield, S. P., Capron, R., Severino, A., Penttila, P. A., Alfred, S., Nahal, H., et al. (2013). Incipient stem cell niche conversion in tissue culture: Using a systems approach to probe early events in *WUSCHEL*-dependent conversion of lateral root primordia into shoot meristems. *The Plant Journal*, *73*, 798–813.

Che, P., Gingerich, D. J., Lall, S., & Howell, S. H. (2002). Global and hormone-induced gene expression changes during shoot development in *Arabidopsis*. *Plant Cell*, *14*, 2771–2785.

Che, P., Lall, S., & Howell, S. H. (2007). Developmental steps in acquiring competence for shoot development in *Arabidopsis* tissue culture. *Planta*, *226*, 1183–1194.
Che, P., Lall, S., & Howell, S. H. (2008). Acquiring competence for shoot development in *Arabidopsis*: ARR2 directly targets A-type *ARR* genes that are differentially activated by CIM preincubation. *Plant Signaling & Behavior*, *3*, 99–101.
Che, P., Lall, S., Nettleton, D., & Howell, S. H. (2006). Gene expression programs during shoot, root, and callus development in Arabidopsis tissue culture. *Plant Physiology*, *141*, 620–637.
Chen, D., Molitor, A., Liu, C., & Shen, W. H. (2010). The *Arabidopsis* PRC1-like ring-finger proteins are necessary for repression of embryonic traits during vegetative growth. *Cell Research*, *20*, 1332–1344.
Cheng, Z. J., Wang, L., Sun, W., Zhang, Y., Zhou, C., Su, Y. H., et al. (2013). Pattern of auxin and cytokinin responses for shoot meristem induction results from the regulation of cytokinin biosynthesis by AUXIN RESPONSE FACTOR3. *Plant Physiology*, *161*, 240–251.
Cheng, Z. J., Zhu, S. S., Gao, X. Q., & Zhang, X. S. (2010). Cytokinin and auxin regulates *WUS* induction and inflorescence regeneration in vitro in *Arabidopsis*. *Plant Cell Reports*, *29*, 927–933.
Clapier, C. R., & Cairns, B. R. (2009). The biology of chromatin remodeling complexes. *Annual Review of Biochemistry*, *78*, 273–304.
Correa Lda, R., Troleis, J., Mastroberti, A. A., Mariath, J. E., & Fett-Neto, A. G. (2012). Distinct modes of adventitious rooting in *Arabidopsis thaliana*. *Plant Biology (Stuttgart, Germany)*, *14*, 100–109.
Curaba, J., Moritz, T., Blervaque, R., Parcy, F., Raz, V., Herzog, M., et al. (2004). *AtGA3ox2*, a key gene responsible for bioactive gibberellin biosynthesis, is regulated during embryogenesis by *LEAFY COTYLEDON2* and *FUSCA3* in Arabidopsis. *Plant Physiology*, *136*, 3660–3669.
de Almeida, M., de Almeida, C. V., Mendes Graner, E., Ebling Brondani, G., & Fiori de Abreu-Tarazi, M. (2012). Pre-procambial cells are niches for pluripotent and totipotent stem-like cells for organogenesis and somatic embryogenesis in the peach palm: A histological study. *Plant Cell Reports*, *31*, 1495–1515.
de Castro, R. D., & Hilhorst, H. W. M. (2006). Hormonal control of seed development in GA- and ABA-deficient tomato (*Lycopersicon esculentum* Mill. cv. Moneymaker) mutants. *Plant Science*, *170*, 462–470.
Dean Rider, S., Jr., Henderson, J. T., Jerome, R. E., Edenberg, H. J., Romero-Severson, J., & Ogas, J. (2003). Coordinate repression of regulators of embryonic identity by *PICKLE* during germination in *Arabidopsis*. *The Plant Journal*, *35*, 33–43.
Duclercq, J., Assoumou Ndong, Y. P., Guerineau, F., Sangwan, R. S., & Catterou, M. (2011). *Arabidopsis* shoot organogenesis is enhanced by an amino acid change in the ATHB15 transcription factor. *Plant Biology (Stuttgart, Germany)*, *13*, 317–324.
Duclercq, J., Sangwan-Norreel, B., Catterou, M., & Sangwan, R. S. (2011). *De novo* shoot organogenesis: From art to science. *Trends in Plant Science*, *16*, 597–606.
Elhiti, M., Hebelstrup, K. H., Wang, A., Li, C., Cui, Y., Hill, R. D., et al. (2013). Function of type-2 Arabidopsis hemoglobin in the auxin-mediated formation of embryogenic cells during morphogenesis. *The Plant Journal*, *74*, 946–958.
Elhiti, M., Tahir, M., Gulden, R. H., Khamiss, K., & Stasolla, C. (2010). Modulation of embryo-forming capacity in culture through the expression of Brassica genes involved in the regulation of the shoot apical meristem. *Journal of Experimental Botany*, *61*, 4069–4085.
Elo, A., Immanen, J., Nieminen, K., & Helariutta, Y. (2009). Stem cell function during plant vascular development. *Seminars in Cell & Developmental Biology*, *20*, 1097–1106.

Fan, M., Xu, C., Xu, K., & Hu, Y. (2012). LATERAL ORGAN BOUNDARIES DOMAIN transcription factors direct callus formation in *Arabidopsis* regeneration. *Cell Research*, *22*, 1169–1180.

Feldman, L. J. (1976). The *de novo* origin of the quiescent center in regenerating root apices of *Zea mays*. *Planta*, *128*, 207–212.

Feng, Z., Sun, X., Wang, G., Liu, H., & Zhu, J. (2012). LBD29 regulates the cell cycle progression in response to auxin during lateral root formation in *Arabidopsis thaliana*. *Annals of Botany*, *110*, 1–10.

Feng, Z., Zhu, J., Du, X., & Cui, X. (2012). Effects of three auxin-inducible LBD members on lateral root formation in *Arabidopsis thaliana*. *Planta*, *236*, 1227–1237.

Furuta, K., Kubo, M., Sano, K., Demura, T., Fukuda, H., Liu, Y. G., et al. (2011). The CKH2/PKL chromatin remodeling factor negatively regulates cytokinin responses in Arabidopsis calli. *Plant & Cell Physiology*, *52*, 618–628.

Gaj, M. D. (2004). Factors influencing somatic embryogenesis induction and plant regeneration with particular reference to *Arabidopsis thaliana* (L.) Heynh. *Plant Growth Regulation*, *43*, 27–47.

Gaudin, V., Libault, M., Pouteau, S., Juul, T., Zhao, G., Lefebvre, D., et al. (2001). Mutations in *LIKE HETEROCHROMATIN PROTEIN 1* affect flowering time and plant architecture in *Arabidopsis*. *Development*, *128*, 4847–4858.

Gautheret, R. J. (1983). Plant tissue culture: A history. *Bot. Mat. Tokyo*, *96*, 393–410.

Gazzarrini, S., Tsuchiya, Y., Lumba, S., Okamoto, M., & McCourt, P. (2004). The transcription factor FUSCA3 controls developmental timing in *Arabidopsis* through the hormones gibberellin and abscisic acid. *Developmental Cell*, *7*, 373–385.

Giraudat, J., Hauge, B. M., Valon, C., Smalle, J., Parcy, F., & Goodman, H. M. (1992). Isolation of the *Arabidopsis ABI3* gene by positional cloning. *Plant Cell*, *4*, 1251–1261.

Goodrich, J., Puangsomlee, P., Martin, M., Long, D., Meyerowitz, E. M., & Coupland, G. (1997). A polycomb-group gene regulates homeotic gene expression in *Arabidopsis*. *Nature*, *386*, 44–51.

Gordon, S. P., Chickarmane, V. S., Ohno, C., & Meyerowitz, E. M. (2009). Multiple feedback loops through cytokinin signaling control stem cell number within the *Arabidopsis* shoot meristem. *Proceedings of the National Academy of Sciences of the United States of America*, *106*, 16529–16534.

Gordon, S. P., Heisler, M. G., Reddy, G. V., Ohno, C., Das, P., & Meyerowitz, E. M. (2007). Pattern formation during de novo assembly of the *Arabidopsis* shoot meristem. *Development*, *134*, 3539–3548.

Greenwood, M. S., Cui, X., Xu, F. (2011). Response to auxin changes during maturation-related loss of adventitious rooting competence in loblolly pine (*Pinus taeda*) stem cuttings. *Physiologia Plantarum, 111*, 373–380.

Harding, E. W., Tang, W., Nichols, K. W., Fernandez, D. E., & Perry, S. E. (2003). Expression and maintenance of embryogenic potential is enhanced through constitutive expression of *AGAMOUS-Like 15*. *Plant Physiology*, *133*, 653–663.

Hays, D., Mandel, R., & Pharis, R. (2001). Hormones in zygotic and microspore embryos of *Brassica napus*. *Plant Growth Regulation*, *35*, 47–58.

He, C., Chen, X., Huang, H., & Xu, L. (2012). Reprogramming of H3K27me3 is critical for acquisition of pluripotency from cultured *Arabidopsis* tissues. *PLoS Genetics*, *8*, e1002911.

Hecht, V., Vielle-Calzada, J. P., Hartog, M. V., Schmidt, E. D., Boutilier, K., Grossniklaus, U., et al. (2001). The Arabidopsis *SOMATIC EMBRYOGENESIS RECEPTOR KINASE 1* gene is expressed in developing ovules and embryos and enhances embryogenic competence in culture. *Plant Physiology*, *127*, 803–816.

Heck, G. R., Perry, S. E., Nichols, K. W., & Fernandez, D. E. (1995). AGL15, a MADS domain protein expressed in developing embryos. *Plant Cell*, *7*, 1271–1282.

Hu, J., Mitchum, M. G., Barnaby, N., Ayele, B. T., Ogawa, M., Nam, E., et al. (2008). Potential sites of bioactive gibberellin production during reproductive growth in *Arabidopsis*. *Plant Cell*, *20*, 320–336.

Hwang, I., & Sheen, J. (2001). Two-component circuitry in *Arabidopsis* cytokinin signal transduction. *Nature*, *413*, 383–389.

Ikeda, Y., Banno, H., Niu, Q. W., Howell, S. H., & Chua, N. H. (2006). The *ENHANCER OF SHOOT REGENERATION 2* gene in *Arabidopsis* regulates *CUP-SHAPED COTYLEDON 1* at the transcriptional level and controls cotyledon development. *Plant & Cell Physiology*, *47*, 1443–1456.

Ikeda-Iwai, M., Satoh, S., & Kamada, H. (2002). Establishment of a reproducible tissue culture system for the induction of *Arabidopsis* somatic embryos. *Journal of Experimental Botany*, *53*, 1575–1580.

Inoue, T., Higuchi, M., Hashimoto, Y., Seki, M., Kobayashi, M., Kato, T., et al. (2001). Identification of CRE1 as a cytokinin receptor from *Arabidopsis*. *Nature*, *409*, 1060–1063.

Ishikawa, M., Murata, T., Sato, Y., Nishiyama, T., Hiwatashi, Y., Imai, A., et al. (2011). *Physcomitrella* cyclin-dependent kinase A links cell cycle reactivation to other cellular changes during reprogramming of leaf cells. *Plant Cell*, *23*, 2924–2938.

Iwase, A., Mitsuda, N., Koyama, T., Hiratsu, K., Kojima, M., Arai, T., et al. (2011). The AP2/ERF transcription factor WIND1 controls cell dedifferentiation in *Arabidopsis*. *Current Biology*, *21*, 508–514.

Iwase, A., Ohme-Takagi, M., & Sugimoto, K. (2011). WIND1: A key molecular switch for plant cell dedifferentiation. *Plant Signaling & Behavior*, *6*, 1943–1945.

Jimenez, V. M. (2005). Involvement of plant hormones and plant growth regulators on *in vitro* somatic embryogenesis. *Plant Growth Regulation*, *47*, 91–110.

Kagaya, Y., Okuda, R., Ban, A., Toyoshima, R., Tsutsumida, K., Usui, H., et al. (2005). Indirect ABA-dependent regulation of seed storage protein genes by FUSCA3 transcription factor in *Arabidopsis*. *Plant & Cell Physiology*, *46*, 300–311.

Kagaya, Y., Toyoshima, R., Okuda, R., Usui, H., Yamamoto, A., & Hattori, T. (2005). *LEAFY COTYLEDON1* controls seed storage protein genes through its regulation of *FUSCA3* and *ABSCISIC ACID INSENSITIVE3*. *Plant & Cell Physiology*, *46*, 399–406.

Karlova, R., Boeren, S., Russinova, E., Aker, J., Vervoort, J., & de Vries, S. (2006). The Arabidopsis SOMATIC EMBRYOGENESIS RECEPTOR-LIKE KINASE1 protein complex includes BRASSINOSTEROID-INSENSITIVE1. *Plant Cell*, *18*, 626–638.

Kotake, T., Takada, S., Nakahigashi, K., Ohto, M., & Goto, K. (2003). *Arabidopsis TERMINAL FLOWER 2* gene encodes a heterochromatin protein 1 homolog and represses both *FLOWERING LOCUS T* to regulate flowering time and several floral homeotic genes. *Plant & Cell Physiology*, *44*, 555–564.

Kwong, R. W., Bui, A. Q., Lee, H., Kwong, L. W., Fischer, R. L., Goldberg, R. B., et al. (2003). *LEAFY COTYLEDON1-LIKE* defines a class of regulators essential for embryo development. *Plant Cell*, *15*, 5–18.

Laplaze, L., Parizot, B., Baker, A., Ricaud, L., Martiniere, A., Auguy, F., et al. (2005). GAL4-GFP enhancer trap lines for genetic manipulation of lateral root development in *Arabidopsis thaliana*. *Journal of Experimental Botany*, *56*, 2433–2442.

Lee, H. W., Kim, M. J., Kim, N. Y., Lee, S. H., & Kim, J. (2013). LBD18 acts as a transcriptional activator that directly binds to the *EXPANSIN14* promoter in promoting lateral root emergence of Arabidopsis. *The Plant Journal*, *73*, 212–224.

Leibfried, A., To, J. P., Busch, W., Stehling, S., Kehle, A., Demar, M., et al. (2005). WUSCHEL controls meristem function by direct regulation of cytokinin-inducible response regulators. *Nature*, *438*, 1172–1175.

Leon, J., Rojo, E., & Sanchez-Serrano, J. J. (2001). Wound signalling in plants. *Journal of Experimental Botany*, *52*, 1–9.

Li, W., Liu, H., Cheng, Z. J., Su, Y. H., Han, H. N., Zhang, Y., et al. (2011). DNA methylation and histone modifications regulate *De Novo* shoot regeneration in *Arabidopsis* by modulating *WUSCHEL* expression and auxin signaling. *PLoS Genetics*, 7, e1002243.

Lotan, T., Ohto, M., Yee, K. M., West, M. A., Lo, R., Kwong, R. W., et al. (1998). *Arabidopsis LEAFY COTYLEDON1* is sufficient to induce embryo development in vegetative cells. *Cell*, *93*, 1195–1205.

Luerssen, H., Kirik, V., Herrmann, P., & Misera, S. (1998). *FUSCA3* encodes a protein with a conserved VP1/AB13-like B3 domain which is of functional importance for the regulation of seed maturation in *Arabidopsis thaliana*. *The Plant Journal*, *15*, 755–764.

Luo, Y., & Koop, H. U. (1997). Somatic embryogenesis in cultured immature zygotic embryos and leaf protoplasts of *Arabidopsis thaliana* ecotypes. *Planta*, *202*, 387–396.

Maffei, M. E., Mithofer, A., & Boland, W. (2007). Before gene expression: Early events in plant-insect interaction. *Trends in Plant Science*, *12*, 310–316.

Makarevich, G., Leroy, O., Akinci, U., Schubert, D., Clarenz, O., Goodrich, J., et al. (2006). Different *Polycomb* group complexes regulate common target genes in *Arabidopsis*. *EMBO Reports*, *7*, 947–952.

Matsuo, N., Makino, M., & Banno, H. (2011). *Arabidopsis ENHANCER OF SHOOT REGENERATION (ESR)1* and *ESR2* regulate *in vitro* shoot regeneration and their expressions are differentially regulated. *Plant Science*, *181*, 39–46.

Michalczuk, L., Ribnicky, D. M., Cooke, T. J., & Cohen, J. D. (1992). Regulation of indole-3-acetic Acid biosynthetic pathways in carrot cell cultures. *Plant Physiology*, *100*, 1346–1353.

Mordhorst, A. P., Hartog, M. V., El Tamer, M. K., Laux, T., & de Vries, S. C. (2002). Somatic embryogenesis from *Arabidopsis* shoot apical meristem mutants. *Planta*, *214*, 829–836.

Ogas, J., Cheng, J. C., Sung, Z. R., & Somerville, C. (1997). Cellular differentiation regulated by gibberellin in the *Arabidopsis thaliana pickle* mutant. *Science*, *277*, 91–94.

Ogas, J., Kaufmann, S., Henderson, J., & Somerville, C. (1999). PICKLE is a CHD3 chromatin-remodeling factor that regulates the transition from embryonic to vegetative development in *Arabidopsis*. *Proceedings of the National Academy of Sciences of the United States of America*, *96*, 13839–13844.

Ogawa, M., Hanada, A., Yamauchi, Y., Kuwahara, A., Kamiya, Y., & Yamaguchi, S. (2003). Gibberellin biosynthesis and response during *Arabidopsis* seed germination. *Plant Cell*, *15*, 1591–1604.

Ohtani, M., Demura, T., & Sugiyama, M. (2008). Differential requirement for the function of SRD2, an snRNA transcription activator, in various stages of plant development. *Plant Molecular Biology*, *66*, 303–314.

Ohtani, M., & Sugiyama, M. (2005). Involvement of SRD2-mediated activation of snRNA transcription in the control of cell proliferation competence in *Arabidopsis*. *The Plant Journal*, *43*, 479–490.

Okita, K., Ichisaka, T., & Yamanaka, S. (2007). Generation of germline-competent induced pluripotent stem cells. *Nature*, *448*, 313–317.

Okushima, Y., Fukaki, H., Onoda, M., Theologis, A., & Tasaka, M. (2007). ARF7 and ARF19 regulate lateral root formation via direct activation of *LBD/ASL* genes in *Arabidopsis*. *Plant Cell*, *19*, 118–130.

Ozawa, S., Yasutani, I., Fukuda, H., Komamine, A., & Sugiyama, M. (1998). Organogenic responses in tissue culture of *srd* mutants of *Arabidopsis thaliana*. *Development*, *125*, 135–142.

Peret, B., De Rybel, B., Casimiro, I., Benkova, E., Swarup, R., Laplaze, L., et al. (2009). *Arabidopsis* lateral root development: An emerging story. *Trends in Plant Science*, *14*, 399–408.

Pernisova, M., Klima, P., Horak, J., Valkova, M., Malbeck, J., Soucek, P., et al. (2009). Cytokinins modulate auxin-induced organogenesis in plants via regulation of the auxin efflux. *Proceedings of the National Academy of Sciences of the United States of America*, *106*, 3609–3614.

Phillips, J., Artsaenko, O., Fiedler, U., Horstmann, C., Mock, H. P., Muntz, K., et al. (1997). Seed-specific immunomodulation of abscisic acid activity induces a developmental switch. *The EMBO Journal*, *16*, 4489–4496.

Pischke, M. S., Huttlin, E. L., Hegeman, A. D., & Sussman, M. R. (2006). A transcriptome-based characterization of habituation in plant tissue culture. *Plant Physiology*, *140*, 1255–1278.

Piyatrakul, P., Putranto, R. A., Martin, F., Rio, M., Dessailly, F., Leclercq, J., et al. (2012). Some ethylene biosynthesis and AP2/ERF genes reveal a specific pattern of expression during somatic embryogenesis in *Hevea brasiliensis*. *BMC Plant Biology*, *12*, 244.

Raghavan, V. (2004). Role of 2,4-dichlorophenoxyacetic acid (2,4-D) in somatic embryogenesis on cultured zygotic embryos of *Arabidopsis*: Cell expansion, cell cycling, and morphogenesis during continuous exposure of embryos to 2,4-D. *American Journal of Botany*, *91*, 1743–1756.

Reid, J. B., & Ross, J. J. (2011). Regulation of tissue repair in plants. *Proceedings of the National Academy of Sciences of the United States of America*, *108*, 17241–17242.

Rost, T. L., Barbour, M. G., Stocking, C. R., & Murphy, T. M. (1997). *Plant biology*. Belmont, California, USA: Wadsworth publishing company.

Rounsley, S. D., Ditta, G. S., & Yanofsky, M. F. (1995). Diverse roles for MADS box genes in Arabidopsis development. *Plant Cell*, *7*, 1259–1269.

Rupp, H. M., Frank, M., Werner, T., Strnad, M., & Schmulling, T. (1999). Increased steady state mRNA levels of the *STM* and *KNAT1* homeobox genes in cytokinin overproducing *Arabidopsis thaliana* indicate a role for cytokinins in the shoot apical meristem. *The Plant Journal*, *18*, 557–563.

Sabatini, S., Beis, D., Wolkenfelt, H., Murfett, J., Guilfoyle, T., Malamy, J., et al. (1999). An auxin-dependent distal organizer of pattern and polarity in the *Arabidopsis* root. *Cell*, *99*, 463–472.

Sablowski, R. (2009). Cytokinin and WUSCHEL tie the knot around plant stem cells. *Proceedings of the National Academy of Sciences of the United States of America*, *106*, 16016–16017.

Sakai, H., Honma, T., Aoyama, T., Sato, S., Kato, T., Tabata, S., et al. (2001). ARR1, a transcription factor for genes immediately responsive to cytokinins. *Science*, *294*, 1519–1521.

Sarkar, A. K., Luijten, M., Miyashima, S., Lenhard, M., Hashimoto, T., Nakajima, K., et al. (2007). Conserved factors regulate signalling in *Arabidopsis thaliana* shoot and root stem cell organizers. *Nature*, *446*, 811–814.

Scheres, B. (2007). Stem-cell niches: Nursery rhymes across kingdoms. *Nature Reviews. Molecular Cell Biology*, *8*, 345–354.

Schubert, D., Primavesi, L., Bishopp, A., Roberts, G., Doonan, J., Jenuwein, T., et al. (2006). Silencing by plant polycomb-group genes requires dispersed trimethylation of histone H3 at lysine 27. *The EMBO Journal*, *25*, 4638–4649.

Sena, G., & Birnbaum, K. D. (2010). Built to rebuild: In search of organizing principles in plant regeneration. *Current Opinion in Genetics & Development*, *20*, 460–465.

Sena, G., Wang, X., Liu, H. Y., Hofhuis, H., & Birnbaum, K. D. (2009). Organ regeneration does not require a functional stem cell niche in plants. *Nature*, *457*, 1150–1153.

Skoog, F., & Miller, C. O. (1957). Chemical regulation of growth and organ formation in plant tissues cultured in vitro. *Symposia of the Society for Experimental Biology*, *11*, 118–130.

Steward, F. C., Mapbs, M. O., & Mears, K. (1958). Growth and organized development of culture cells. II. Organization in cultures grown from freely suspended cells. *American Journal of Botany*, *45*, 705–708.

Stone, S. L., Braybrook, S. A., Paula, S. L., Kwong, L. W., Meuser, J., Pelletier, J., et al. (2008). *Arabidopsis* LEAFY COTYLEDON2 induces maturation traits and auxin activity: Implications for somatic embryogenesis. *Proceedings of the National Academy of Sciences of the United States of America*, *105*, 3151–3156.

Stone, S. L., Kwong, L. W., Yee, K. M., Pelletier, J., Lepiniec, L., Fischer, R. L., et al. (2001). *LEAFY COTYLEDON2* encodes a B3 domain transcription factor that induces embryo development. *Proceedings of the National Academy of Sciences of the United States of America*, *98*, 11806–11811.

Stroud, H., Ding, B., Simon, S. A., Feng, S., Bellizzi, M., Pellegrini, M., et al. (2013). Plants regenerated from tissue culture contain stable epigenome changes in rice. *Elife*, *2*, e00354.

Su, Y. H., & Zhang, X. S. (2009). Auxin gradients trigger de novo formation of stem cells during somatic embryogenesis. *Plant Signaling & Behavior*, *4*, 574–576.

Su, Y. H., Zhao, X. Y., Liu, Y. B., Zhang, C. L., O'Neill, S. D., & Zhang, X. S. (2009). Auxin-induced *WUS* expression is essential for embryonic stem cell renewal during somatic embryogenesis in Arabidopsis. *The Plant Journal*, *59*, 448–460.

Sugimoto, K., Gordon, S. P., & Meyerowitz, E. M. (2011). Regeneration in plants and animals: Dedifferentiation, transdifferentiation, or just differentiation? *Trends in Cell Biology*, *21*, 212–218.

Sugimoto, K., Jiao, Y., & Meyerowitz, E. M. (2010). *Arabidopsis* regeneration from multiple tissues occurs via a root development pathway. *Developmental Cell*, *18*, 463–471.

Sussex, I. M. (2008). The scientific roots of modern plant biotechnology. *Plant Cell*, *20*, 1189–1198.

Suzuki, M., Wang, H. H., & McCarty, D. R. (2007). Repression of the *LEAFY COTYLEDON 1/B3* regulatory network in plant embryo development by *VP1/ABSCISIC ACID INSENSITIVE 3-LIKE* B3 genes. *Plant Physiology*, *143*, 902–911.

Takahashi, K., & Yamanaka, S. (2006). Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell*, *126*, 663–676.

Tamaki, H., Konishi, M., Daimon, Y., Aida, M., Tasaka, M., & Sugiyama, M. (2009). Identification of novel meristem factors involved in shoot regeneration through the analysis of temperature-sensitive mutants of Arabidopsis. *The Plant Journal*, *57*, 1027–1039.

Thorpe, T. A. (2006). History of plant tissue culture. *Methods in Molecular Biology*, *318*, 9–32.

Thorpe, T. A. (2007). History of plant tissue culture. *Molecular Biotechnology*, *37*, 169–180.

Tsuwamoto, R., Yokoi, S., & Takahata, Y. (2010). *Arabidopsis EMBRYOMAKER* encoding an AP2 domain transcription factor plays a key role in developmental change from vegetative to embryonic phase. *Plant Molecular Biology*, *73*, 481–492.

Turck, F., Roudier, F., Farrona, S., Martin-Magniette, M. L., Guillaume, E., Buisine, N., et al. (2007). Arabidopsis TFL2/LHP1 specifically associates with genes marked by trimethylation of histone H3 lysine 27. *PLoS Genetics*, *3*, e86.

Vahdati, K., Bayat, S., Ebrahimzadeh, H., Jariteh, M., & Mirmasoumi, M. (2008). Effect of exogenous ABA on somatic embryo maturation and germination in Persian walnut (Juglans regia L.). *Plant Cell, Tissue and Organ Culture*, *93*, 163–171.

Verdeil, J. L., Alemanno, L., Niemenak, N., & Tranbarger, T. J. (2007). Pluripotent versus totipotent plant stem cells: Dependence versus autonomy? *Trends in Plant Science*, *12*, 245–252.

Wang, H., Caruso, L. V., Downie, A. B., & Perry, S. E. (2004). The embryo MADS domain protein AGAMOUS-Like 15 directly regulates expression of a gene encoding an enzyme involved in gibberellin metabolism. *Plant Cell*, *16*, 1206–1219.

Wang, X., Niu, Q. W., Teng, C., Li, C., Mu, J., Chua, N. H., et al. (2009). Overexpression of *PGA37/MYB118* and *MYB115* promotes vegetative-to-embryonic transition in *Arabidopsis*. *Cell Research*, *19*, 224–235.

White, C. N., Proebsting, W. M., Hedden, P., & Rivin, C. J. (2000). Gibberellins and seed development in *Maize*. I. Evidence that gibberellin/abscisic acid balance governs germination versus maturation pathways. *Plant Physiology*, *122*, 1081–1088.

Xiao, L., Zhang, L., Yang, G., Zhu, H., & He, Y. (2012). Transcriptome of protoplasts reprogrammed into stem cells in *Physcomitrella patens*. *PLoS One*, 7, e35961.

Xu, J., Hofhuis, H., Heidstra, R., Sauer, M., Friml, J., & Scheres, B. (2006). A molecular framework for plant regeneration. *Science*, *311*, 385–388.

Xu, K., Liu, J., Fan, M., Xin, W., Hu, Y., & Xu, C. (2012). A genome-wide transcriptome profiling reveals the early molecular events during callus initiation in *Arabidopsis* multiple organs. *Genomics*, *100*, 116–124.

Xu, L., & Shen, W.-H. (2008). Polycomb silencing of *KNOX* genes confines shoot stem cell niches in *Arabidopsis*. *Current Biology*, *18*, 1966–1971.

Yamaguchi, S., Kamiya, Y., & Nambara, E. (2007). Regulation of ABA and GA levels during seed development and germination in *Arabidopsis*. In K. J. Bradford & H. Nonogaki (Eds.), *Seed development, dormancy and germination* (pp. 224–247). Oxford, UK: Blackwell Publishing.

Yanai, O., Shani, E., Dolezal, K., Tarkowski, P., Sablowski, R., Sandberg, G., et al. (2005). *Arabidopsis* KNOXI proteins activate cytokinin biosynthesis. *Current Biology*, *15*, 1566–1571.

Yang, C., Bratzel, F., Hohmann, N., Koch, M., Turck, F., & Calonje, M. (2013). VAL- and AtBMI1-mediated H2Aub initiate the switch from embryonic to postgerminative growth in *Arabidopsis*. *Current Biology*, *23*, 1324–1329.

Yang, C. H., Chen, L. J., & Sung, Z. R. (1995). Genetic regulation of shoot development in *Arabidopsis*: Role of the *EMF* genes. *Developmental Biology*, *169*, 421–435.

Yang, X. Y., & Zhang, X. L. (2010). Regulation of somatic embryogenesis in higher plants. *Critical Reviews in Plant Sciences*, *29*, 36–57.

Yasutani, I., Ozawa, S., Nishida, T., Sugiyama, M., & Komamine, A. (1994). Isolation of temperature-sensitive mutants of *Arabidopsis thaliana* that are defective in the redifferentiation of shoots. *Plant Physiology*, *105*, 815–822.

Yoshida, N., Yanai, Y., Chen, L., Kato, Y., Hiratsuka, J., Miwa, T., et al. (2001). EMBRYONIC FLOWER2, a novel polycomb group protein homolog, mediates shoot development and flowering in Arabidopsis. *Plant Cell*, *13*, 2471–2481.

Zhang, H., Bishop, B., Ringenberg, W., Muir, W. M., & Ogas, J. (2012). The CHD3 remodeler PICKLE associates with genes enriched for trimethylation of histone H3 lysine 27. *Plant Physiology*, *159*, 418–432.

Zhang, X., Clarenz, O., Cokus, S., Bernatavichute, Y. V., Pellegrini, M., Goodrich, J., et al. (2007). Whole-genome analysis of histone H3 lysine 27 trimethylation in *Arabidopsis*. *PLoS Biology*, *5*, e129.

Zhang, X., Germann, S., Blus, B. J., Khorasanizadeh, S., Gaudin, V., & Jacobsen, S. E. (2007). The *Arabidopsis* LHP1 protein colocalizes with histone H3 Lys27 trimethylation. *Nature Structural & Molecular Biology*, *14*, 869–871.

Zhang, H., Rider, S. D., Jr., Henderson, J. T., Fountain, M., Chuang, K., Kandachar, V., et al. (2008). The CHD3 remodeler PICKLE promotes trimethylation of histone H3 lysine 27. *The Journal of Biological Chemistry*, *283*, 22637–22648.

Zhao, Z., Andersen, S. U., Ljung, K., Dolezal, K., Miotk, A., Schultheiss, S. J., et al. (2010). Hormonal control of the shoot stem-cell niche. *Nature*, *465*, 1089–1092.

Zheng, Y., Ren, N., Wang, H., Stromberg, A. J., & Perry, S. E. (2009). Global identification of targets of the *Arabidopsis* MADS domain protein AGAMOUS-Like15. *Plant Cell*, *21*, 2563–2577.

Zheng, Q., Zheng, Y., & Perry, S. E. (2013). AGAMOUS-Like15 promotes somatic embryogenesis in Arabidopsis and soybean in part by the control of ethylene biosynthesis and response. *Plant Physiology*, *161*, 2113–2127.

Zimmerman, J. L. (1993). Somatic embryogenesis: A model for early development in higher plants. *Plant Cell*, *5*, 1411–1423.

Zuo, J., Niu, Q. W., Frugis, G., & Chua, N. H. (2002). The *WUSCHEL* gene promotes vegetative-to-embryonic transition in *Arabidopsis*. *The Plant Journal*, *30*, 349–359.