REVIEW

Plant Callus: Mechanisms of Induction and Repression

Momoko Ikeuchi, Keiko Sugimoto, and Akira Iwase
RIKEN Center for Sustainable Resource Science, Yokohama 230-0045, Japan
ORCID IDs: 0000-0001-9474-5131 (M.I.); 0000-0002-9209-8230 (K.S.); 0000-0003-3294-7939 (A.I.)

Plants develop unorganized cell masses like callus and tumors in response to various biotic and abiotic stimuli. Since the historical discovery that the combination of two growth-promoting hormones, auxin and cytokinin, induces callus from plant explants in vitro, this experimental system has been used extensively in both basic research and horticultural applications. The molecular basis of callus formation has long been obscure, but we are finally beginning to understand how unscheduled cell proliferation is suppressed during normal plant development and how genetic and environmental cues override these repressions to induce callus formation. In this review, we will first provide a brief overview of callus development in nature and in vitro and then describe our current knowledge of genetic and epigenetic mechanisms underlying callus formation.

INTRODUCTION

Having high plasticity for cell differentiation is one central characteristic of plant cells. Plants generate unorganized cell masses, such as callus or tumors, in response to stresses, such as wounding or pathogen infection. Callus formation in debarked trees was described over 200 years ago (Neely, 1979, and references therein). The term “callus” originates from the Latin word callum, which means hard, and in medicine it refers to the thickening of dermal tissue. “Callus” in the early days of plant biology referred to the massive growth of cells and accumulation of callose associated with wounding. Today the same word is used more broadly, and disorganized cell masses are collectively called callus. Callus can be produced from a single differentiated cell, and many callus cells are totipotent, being able to regenerate the whole plant body (Steward et al., 1958; Nagata and Takebe, 1971). Under certain conditions, callus cells also undergo somatic embryogenesis, a process in which embryos are generated from adult somatic cells (Steward et al., 1958). Thus, at least some forms of callus formation are thought to involve cell dedifferentiation. However, it has also been acknowledged that calli are very diverse and can be classified into subgroups based on their macroscopic characteristics. For example, calli with no apparent organ regeneration typically are called friable or compact callus (Figure 1A). Other calli that display some degrees of organ regeneration are called rooty, shooty, or embryonic callus, depending on the organs they generate (Zimmerman, 1993; Frank et al., 2000) (Figure 1A). It is also known that different types of callus in Arabidopsis thaliana have distinct gene expression profiles (Iwase et al., 2011a). Therefore, the term callus includes cells with various degrees of differentiation.

After the groundbreaking discovery that callus can be generated artificially in vitro (Gautheret, 1939; Nobécourt, 1939; White, 1939) and that the balance between two plant hormones, auxin and cytokinin, determines the state of differentiation and dedifferentiation (Skoog and Miller, 1957), callus has been widely used in both basic research and industrial applications (George and Sherrington, 1984; Bourgaud et al., 2001). However, despite its extensive use, our knowledge of the molecular mechanisms underlying callus formation has been limited until recently. Through the extensive characterization of loss-of-function and gain-of-function mutants with callus phenotypes, we are finally beginning to understand how callus develops in response to various physiological and environmental stimuli. It is also becoming increasingly clear that plants are equipped with a robust mechanism to prevent unwanted callus induction to maintain their tissue organization. In this review, we will first provide an overview of callus and tumor formation in vitro and in nature to highlight the similarities and diversities of their physiological properties. We will then summarize our current knowledge of how plants reprogram their differentiation status and regain proliferative competence to produce callus. Finally, we will describe genetic and epigenetic mechanisms that repress callus induction during postembryonic development in plants.

CALLUS FORMATION IN VITRO AND IN NATURE

Callus Formed under In Vitro Culture Conditions

Exogenous application of auxin and cytokinin induces callus in various plant species. Generally speaking, an intermediate ratio of auxin and cytokinin promotes callus induction, while a high ratio of auxin-to-cytokinin or cytokinin-to-auxin induces root and shoot regeneration, respectively (Skoog and Miller, 1957). Since the discovery of this regeneration system, it has been widely used, for example, in the propagation of economically important traits and the introduction of transgenes. Other hormones, such as brassinosteroids or abscisic acid, also induce callus and in some species may substitute auxin or cytokinin in callus formation (Goren et al., 1979; Hu et al., 2000). However, auxin and
cytokinin have been by far the most extensively used and studied hormones in the context of callus formation and subsequent organ regeneration.

In Arabidopsis, shoot or root explants incubated on auxin- and cytokinin-containing callus-inducing medium (CIM) form callus from pericycle cells adjacent to the xylem poles (Valvekens et al., 1988; Atta et al., 2009) (Figures 1B and 2A). Careful histological examination revealed, unexpectedly, that these calli are not a mass of unorganized cells; instead, they have organized structures resembling the primordia of lateral roots (Atta et al., 2009). It was later confirmed by transcriptome analysis that these calli have gene expression profiles highly similar to that of root meristems (Sugimoto et al., 2010) (Figure 1B). Strikingly, even calli generated from aerial organs, such as cotyledons and petals, possess organized structures similar to lateral root primordia (Sugimoto et al., 2010). Consistent with these findings, the formation of CIM-induced callus, irrespective of its origin, is strongly suppressed in aberrant lateral root formation mutants defective in the development of lateral root primordia (Sugimoto et al., 2010). These data collectively suggest that CIM induces callus through the genetic pathway mediating lateral root initiation and that CIM-induced callus, at least in Arabidopsis, is not as dedifferentiated as previously thought.

**Callus Induced by Wounding**

Wound-induced callus formation has long been observed and used in various contexts from debarking of trees (Stobbe et al., 2002) to horticultural use of propagation (Cline and Neely, 1983). These calli often accumulate phytoalexins and pathogen-related proteins (Bostock and Stermer, 1989) and thus are thought to prevent infection as well as water loss. Wound-induced callus derive from various cell types, including vascular cells, cortical cells, and pith cells. In some cases, wound-induced calli regenerate new organs or new tissues, suggesting that they are highly pluripotent (Stobbe et al., 2002).

Wounding promotes callus formation in various parts of Arabidopsis seedlings (Iwase et al., 2011a). As shown in Figures 2A and 2B, the appearance of callus is distinct from CIM-induced callus. In addition, unlike CIM-induced callus, wound-induced callus does not display expression of root meristem markers and its formation is not blocked in solitary root mutants defective in lateral root initiation (Iwase et al., 2011a) (Figure 1B). These observations strongly suggest that these two types of callus are different in their molecular and physiological properties. As we will discuss in more detail later, at least some aspects of wound-induced callus formation are driven through the upregulation of cytokinin signaling (Iwase et al., 2011a).

**Tumors Induced by Pathogens**

Crown gall is a plant disease caused by gram-negative bacteria Agrobacterium tumefaciens (recently renamed as Rhizobium rhizogenes), and it occurs in thousands of plant species (Figure 2C). These bacteria enter plants through wound sites and promote tumorous outgrowth of an unorganized cell mass (Nester et al., 1984). The expression of bacterial genes encoding biosynthetic enzymes of auxin and cytokinin forces infected plants to produce galls. These include tumor morphology shoot1 (tms1), encoding a Trp monoxygenase, and tms2, encoding an indoleacetamide hydrolase involved in the production of auxin (Sitbon et al., 1991), as well as tumor morphology root, encoding an isopentenyl transferase required for the cytokinin production (Akiyoshi et al., 1983, 1984). All of these genes are located on the T-DNA region of the bacterial tumor-inducing plasmid, which is randomly inserted into the genome of host plants upon infection. Crown gall cells can be subcultured without exogenous plant hormones even after the removal of bacteria. In addition, a single cell derived from crown gall can regenerate whole plants (Braun, 1959; Sacristan and Melchers, 1977), indicating that crown gall cells are totipotent. Other gram-negative bacteria, such as Pantoea agglomerans pv gypsophilae and P. agglomerans pv betae, also infect plants and induce gall formation (Figure 2D).
Many of these bacteria produce auxin and cytokinin (Morris, 1986; Glick, 1995) to promote tumorization in host plants (Manulis et al., 1998). In some bacterial species, effector proteins synthesized in bacteria also stimulate gall formation (Barash and Manulis-Sasson, 2007, and references therein).

Viral infection is another source of plant tumorization in nature. The wound tumor viruses (WTVs), also called clover big vein viruses, belong to the family of Group III viruses with the double-stranded RNA genome and induce gall formation in host plants. WTVs induce relatively well organized tumors, consisting of abnormal xylem, meristematic tumor cells, and pseudophloem that are surrounded by cortex and epidermal cells of the host plant (Lee, 1955) (Figure 2E). The rice gall dwarf viruses, which also belong to the family of Group III viruses, induce gall formation in Poaceae species, for example, Oryza sativa (rice), Triticum aestivum (wheat), and Hordeum vulgare (barley). The double-stranded RNA of both WTVs and rice gall dwarf viruses consists of 12 segments, each of which is thought to encode one protein (Zhang et al., 2007, and references therein). Further functional analyses of these proteins should help elucidate the powerful strategies taken by these viruses to intervene with normal plant development.

Gall formation caused by other pathogenic organisms has also been well documented. These include, for instance, club root formation by parasitic protists, such as phytomyxea (Malinowski et al., 2012), root-knot disease by nematodes (Jammes et al., 2005), and gall formation by insects (Tooker et al., 2008). All of these abnormal outgrowth cause serious damage to agricultural crops, but the underpinning molecular mechanisms remain largely unknown.

### Genetic Tumors Induced by Interspecific Hybrids

Genetic tumors refer to unorganized overproliferation of cells that occurs as a result of interspecific crosses and are particularly common in Brassica, Datura, Lilium, and Nicotiana (Ahuja, 1965, and references therein) (Figure 2F). The tumorous cells excised from hybrid plants can be subcultured in phytohormone-free media and exhibit totipotency (White, 1939; Ichikawa and Syōno, 1988). Senescence and wounding further enhance tumorization within the hybrid plants (Udagawa et al., 2004). Molecular mechanisms underlying genetic tumors are not well understood, but the level of endogenous auxin and cytokinin seem to be altered in tumorous hybrid plants (Kehr, 1951; Kung, 1989; Ichikawa and Syōno, 1991). Some genetic tumors are accompanied by misexpression of key regulators in embryogenesis or meristem development (Chiappetta et al., 2006, 2009). Therefore, tumorization might be caused through the reacquisition of undifferentiated status or failure in tissue differentiation.

### MOLECULAR BASIS OF CALLUS FORMATION

Many mutants impaired in callus formation have been identified over the last decade, and molecular genetic analyses of these mutants have revealed that callus induction is governed through complex regulatory mechanisms (Table 1). The progression of the mitotic cell cycle is suppressed in terminally differentiated plant cells, pointing to the reacquisition of cell proliferative competence as a central feature of callus induction. Activation of a single core cell cycle regulator, such as cyclins (CYCs) or cyclin-dependent kinases (CDKs), alone is usually not sufficient to induce callus (Riou-Khamlichi et al., 1999; Cockcroft et al., 2000; Dewitte et al., 2003). Accordingly, most callus induction processes described to date employ transcriptional or post-transcriptional regulators that cause global changes in gene
expression or protein translation. In the next section, we will describe how plants interpret various physiological and environmental signals to trigger cells to reenter the cell cycle.

**Callus Induction by Plant Hormones**

Auxin and cytokinin have been widely used to generate callus, but surprisingly little is known about how they induce callus at the molecular level. Several recent studies demonstrated that various regulators of lateral root development participate in callus formation on CIM. Auxin is a well-known inducer of lateral root formation in *Arabidopsis*, and several members of the LATERAL ORGAN BOUNDARIES DOMAIN (LBD; also known as ASYMMETRIC LEAVES2-LIKE) family of transcription factors, including LBD16, LBD17, LBD18, and LBD29, mediate this response downstream of AUXIN RESPONSE FACTOR7 (ARF7) and ARF19 (Okushima et al., 2007; Lee et al., 2009). A recent study by Berckmans et al. (2011) has provided a first glimpse of how auxin promotes cell cycle reentry during lateral root development by demonstrating that LBD18 and LBD33, both of which are induced by auxin and form a heterodimer complex, activate the expression of the transcription factor E2 PROMOTER.

### Table 1. List of Genes Implicated in Callus Induction or Repression in *Arabidopsis*

<table>
<thead>
<tr>
<th>Locus</th>
<th>Common Name</th>
<th>Protein Family</th>
<th>Predicted Function</th>
<th>References</th>
</tr>
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<tr>
<td>AT2G42430</td>
<td>LBD16</td>
<td>LOB-domain transcription factor (TF)</td>
<td>Auxin response/lateral root formation</td>
<td>Fan et al. (2012)</td>
</tr>
<tr>
<td>AT2G42440</td>
<td>LBD17</td>
<td>LOB-domain TF</td>
<td>Auxin response</td>
<td>Fan et al. (2012)</td>
</tr>
<tr>
<td>AT2G45420</td>
<td>LBD18</td>
<td>LOB-domain TF</td>
<td>Auxin response/lateral root formation</td>
<td>Fan et al. (2012)</td>
</tr>
<tr>
<td>AT3G58190</td>
<td>LBD29</td>
<td>LOB-domain TF</td>
<td>Auxin response/lateral root formation</td>
<td>Fan et al. (2012)</td>
</tr>
<tr>
<td>AT3G18657</td>
<td>ARR1</td>
<td>GARP TF</td>
<td>Cytokinin response</td>
<td>Sakai et al. (2001)</td>
</tr>
<tr>
<td>AT3G07210</td>
<td>ARR21</td>
<td>GARP TF</td>
<td>Cytokinin response</td>
<td>Tajima et al. (2004)</td>
</tr>
<tr>
<td>AT1G12980</td>
<td>ESR1/DRN</td>
<td>AP2/ERF TF</td>
<td>Cytokinin response/shoot regeneration</td>
<td>Banno et al. (2001)</td>
</tr>
<tr>
<td>AT1G24590</td>
<td>ESR2/DRNL/BOL</td>
<td>AP2/ERF TF</td>
<td>Cytokinin response/shoot regeneration</td>
<td>Ikeda et al. (2006); Marsch-Martinez et al. (2006)</td>
</tr>
<tr>
<td>AT1G78080</td>
<td>WIND1/RAP2.4b</td>
<td>AP2/ERF TF</td>
<td>Wound-induced cell dedifferentiation</td>
<td>Iwase et al. (2011a, 2011b)</td>
</tr>
<tr>
<td>AT1G22190</td>
<td>WIND2/RAP2.4d</td>
<td>AP2/ERF TF</td>
<td>Wound-induced cell dedifferentiation</td>
<td>Iwase et al. (2011a, 2011b)</td>
</tr>
<tr>
<td>AT1G36060</td>
<td>WIND3/RAP2.4a</td>
<td>AP2/ERF TF</td>
<td>Wound-induced cell dedifferentiation</td>
<td>Iwase et al. (2011a, 2011b)</td>
</tr>
<tr>
<td>AT5G65130</td>
<td>WIND4</td>
<td>AP2/ERF TF</td>
<td>Wound-induced cell dedifferentiation</td>
<td>Iwase et al. (2011a, 2011b)</td>
</tr>
<tr>
<td>AT1G21970</td>
<td>LEC1</td>
<td>CCAAT-box binding TF</td>
<td>Embryogenesis</td>
<td>Lotan et al. (1998)</td>
</tr>
<tr>
<td>AT1G28300</td>
<td>LEC2</td>
<td>B3 domain TF</td>
<td>Embryogenesis</td>
<td>Stone et al. (2001)</td>
</tr>
<tr>
<td>AT5G37900</td>
<td>AGL15</td>
<td>MADS box TF</td>
<td>Embryogenesis</td>
<td>Harding et al. (2003)</td>
</tr>
<tr>
<td>AT5G17430</td>
<td>BBM</td>
<td>AP2/ERF TF</td>
<td>Embryogenesis</td>
<td>Boutilier et al. (2002)</td>
</tr>
<tr>
<td>AT5G73900</td>
<td>EMK/AIL5/PLT5</td>
<td>AP2/ERF TF</td>
<td>Embryogenesis</td>
<td>Tsuwaramoto et al. (2010)</td>
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<tr>
<td>AT1G18700</td>
<td>RKD1</td>
<td>RWP-RK domain TF</td>
<td>Gametogenesis</td>
<td>Köszegi et al. (2011)</td>
</tr>
<tr>
<td>AT1G47440</td>
<td>RKD2</td>
<td>RWP-RK domain TF</td>
<td>Gametogenesis</td>
<td>Köszegi et al. (2011)</td>
</tr>
<tr>
<td>AT5G53040</td>
<td>RKD4</td>
<td>RWP-RK domain TF</td>
<td>Embryogenesis</td>
<td>Waki et al. (2011)</td>
</tr>
<tr>
<td>AT2G17950</td>
<td>WUS</td>
<td>Homeodomain TF</td>
<td>Stem cell maintenance</td>
<td>Zuo et al. (2002)</td>
</tr>
<tr>
<td>AT3G50380</td>
<td>KRP2</td>
<td>CDK inhibitor</td>
<td>Negative regulation of cell proliferation</td>
<td>Anzola et al. (2010)</td>
</tr>
<tr>
<td>AT5G48820</td>
<td>KRP3</td>
<td>CDK inhibitor</td>
<td>Negative regulation of cell proliferation</td>
<td>Anzola et al. (2010)</td>
</tr>
<tr>
<td>AT1G49620</td>
<td>KRP7</td>
<td>CDK inhibitor</td>
<td>Negative regulation of cell proliferation</td>
<td>Anzola et al. (2010)</td>
</tr>
<tr>
<td>AT5G97200</td>
<td>TSD1/KOR1/RSW2</td>
<td>Endo-1,4-β-c-glucanase</td>
<td>Cellulose biosynthesis (?)</td>
<td>Frank et al. (2002); Crupková and Schmülling (2009)</td>
</tr>
<tr>
<td>AT1G78240</td>
<td>TSD2/QUA2/OSU1</td>
<td>S-adenosyl-L-Met-dependent methyltransferase</td>
<td>Pectin biosynthesis (?)</td>
<td>Frank et al. (2002); Crupková et al. (2007)</td>
</tr>
<tr>
<td>AT4G16845</td>
<td>VRN2</td>
<td>PRC2</td>
<td>Histone H3 Lys-27 trimethylation</td>
<td>Chinni et al. (2004); Schubert et al. (2005)</td>
</tr>
<tr>
<td>AT5G51230</td>
<td>EMF2</td>
<td>PRC2</td>
<td>Histone H3 Lys-27 trimethylation</td>
<td>Chinni et al. (2004); Schubert et al. (2005)</td>
</tr>
<tr>
<td>AT3G20740</td>
<td>FIE</td>
<td>PRC2</td>
<td>Histone H3 Lys-27 trimethylation</td>
<td>Bouyer et al. (2011)</td>
</tr>
<tr>
<td>AT2G30580</td>
<td>AT BMI1A</td>
<td>PRC1</td>
<td>Histone H2A Lys-119 ubiquitination</td>
<td>Bratzel et al. (2010)</td>
</tr>
<tr>
<td>AT1G06770</td>
<td>AT BMI1B</td>
<td>PRC1</td>
<td>Histone H2A Lys-119 ubiquitination</td>
<td>Bratzel et al. (2010)</td>
</tr>
<tr>
<td>AT2G25170</td>
<td>KKL</td>
<td>CHD3/4-like chromatin remodeling factor</td>
<td>Histone H3 Lys-27 trimethylation and histone deacetylation (?)</td>
<td>Ogas et al. (1997, 1999)</td>
</tr>
<tr>
<td>AT2G30470</td>
<td>VAL1/HSI2</td>
<td>B3 domain TF</td>
<td>Termination of embryogenesis</td>
<td>Tsukagoshi et al. (2007)</td>
</tr>
<tr>
<td>AT4G32010</td>
<td>VAL2/HSL1</td>
<td>B3 domain TF</td>
<td>Termination of embryogenesis</td>
<td>Tsukagoshi et al. (2007)</td>
</tr>
</tbody>
</table>

*Genes that promote callus formation upon overexpression.

*Genes that are required to repress callus formation.
BINDING FACTOR a (E2Fa). E2Fa is one of the six E2F transcription factors in Arabidopsis that by dimerizing with DIMERIZATION PARTNER (DP) proteins, promotes the transcription of genes required for DNA replication (Inzé and De Veylder, 2006). The loss-of-function mutation in E2Fa strongly impedes lateral root development; hence, the ARF-LBD-E2Fa pathway defines one mechanism of how plants translate auxin signaling into cell cycle control.

Fan et al. (2012) have shown that the expression of LBD16, LBD17, LBD18, and LBD29 is upregulated by CIM and that overexpression of each of the four is sufficient to induce callus with a similar appearance to CIM-induced callus (Figure 3A). The authors further demonstrated that CIM-induced callus formation is impaired in the arf7 arf19 double mutant, but overexpression of LBD16 in arf7 arf19 allows callus induction, suggesting that these LBDs function downstream of ARF7 and ARF19 (Figure 4A). Functional roles of LBDs appear to be conserved in trees since a LBD homolog in poplar (Populus tremula × Populus alba), Pta-LBD1, also promotes callus formation under low auxin conditions where control plants do not form callus (Yordanov et al., 2010). It is worth noting that overexpression of E2Fa together with DPa enhances cell proliferation in Arabidopsis leaves but not to the extent to induce callus (De Veylder et al., 2002). This might be due to the relatively mild E2Fa/DPa expression in the transgenic plants, but alternatively, LBDs may be needed to activate transcription of additional genes that, together with E2Fa/DPa,

Figure 3. Gain-of-Function and Loss-of-Function Mutants Exhibiting Ectopic Callus Formation in Arabidopsis.

(A) Friable callus generated on the root overexpressing the LBD16 gene.
(B) Friable callus growing around the shoot apex of the KRP silencing plants with reduced levels of KRP2, KRP3, and KRP7 (Anzola et al., 2010).
(C) Friable callus on the hypocotyl and root overexpressing the constitutive active form of the ARR21 gene (Tajima et al., 2004).
(D) Compact callus induced on the ESR1-overexpressing seedling (Banno et al., 2001).
(E) Friable cells growing on the shoot, hypocotyl, and root of WIN1-overexpressing plants (Iwase et al., 2011a).
(F) Somatic embryos generated on WIN1-overexpressing callus.
(G) Embryonic cells induced by the LEC2 overexpression (Stone et al., 2001).
(H) Friable callus generated on the root of RKD4-overexpressing plants (Waki et al., 2011).
(I) Embryonic callus on WUS-overexpressing plants (Zuo et al., 2002).
(J) Friable callus generated by the tsd1 loss-of-function mutation (Krupková and Schmülling, 2009).
(K) Embryonic and rooty callus in the clf swn double mutant (Chanvivattana et al., 2004). Arrows indicate root hairs developing from the callus.
(L) Embryonic and rooty callus in the At bmi1a and At bmi1b double mutant (Bratzel et al., 2010). All plants shown here are grown on phytohormone-free medium.

Bars = 1 mm in (A), (B), (E), (G), and (I) to (K), 5 mm in (D), 500 μm in (H), and 2 mm in (L). Image in [B] is reprinted from Anzola et al. [2010], Supplemental Figure 5E with permission from National Academy of Sciences; [C] is reprinted from Tajima et al. [2004], Figure 6C with permission from Oxford University Press; [D] is reprinted from Banno et al. [2001], Figure 5B; [G] is reprinted from Stone et al. [2001], Figure 5D with permission from National Academy of Sciences; [H] is reprinted from Waki et al. [2011], Figure 4I with permission from Cell Press; [I] is reprinted from Zuo et al. [2002], Figure 2C with permission from John Wiley and Sons; [J] is reprinted from Krupková and Schmülling [2009], Figure 1A with permission from Springer; [K] is reprinted from Chanvivattana et al. [2004], Figure 3H with permission from Company of Biologists; [L] is reprinted from Bratzel et al. [2011], Figure 2J with permission from Cell Press.)
promote callus induction. Overexpression of E2Fa and DP causes similar overproliferation in tobacco (Nicotiana tabacum) leaves, and, interestingly, it also promotes callus formation at the wound site (Kosugi and Ohashi, 2003). These observations support the notion that callus induction requires activation of both E2Fa/DP and some other factors, in this case, produced by wounding.

Besides activating core cell cycle regulators, downregulation of cell cycle inhibitors is another strategy for the reacquisition of cell proliferative competence during callus formation. Auxin downregulates the KIP-RELATED PROTEIN (KRP) genes encoding CDK inhibitors, and a transcriptional adaptor protein PROPRZ1 (PRZ1, also known as At-ADA2b) has been identified as a key regulator in this process (Anzola et al., 2010) (Figure 4A). The prz1 roots develop callus in the hormonal condition where wild-type roots form lateral roots, and this overproliferation is accompanied by low transcript levels of KRP2, KRP3, and KRP7 (Sieberer et al., 2003). PRZ1 directly binds the promoter region of KRP2, KRP3, and KRP7 and promotes acetylation of histone H3-K9/K14 at KRP7. The acetylation level decreases in response to auxin treatment, which in turn reduces gene expression (Anzola et al., 2010). Callus formation was phenocopied in the KRP silencing lines with reduced levels of KRP2, KRP3, and KRP7 (Figure 3B), whereas overexpression of KRP7 partially antagonizes the overproliferation phenotype in prz1 (Anzola et al., 2010). These findings thus demonstrate that the PRZ1-dependent chromatin modification provides an additional molecular mechanism of decoding auxin signaling into cell cycle reactivation.

How cytokinin promotes callus formation is less clear, but a critical component that participates in callus induction is the type-B ARABIDOPSIS RESPONSE REGULATORS (ARRs) (Figure 4B). The type-B ARRs transcription factors are activated through a multistep phosphorylation and induce the expression of many target genes (Hwang et al., 2012). Overexpression of ARR1 in cytokinin-containing media enhances callus formation in Arabidopsis (Sakai et al., 2001), thus elevating the fact that ARR1-mediated cytokinin response is sufficient to induce callus. In support of this idea, overexpression of the constitutively active form of ARR1 or ARR21, lacking the phosphorylation domain, results in callus formation in the absence of exogenous plant hormones (Sakai et al., 2001; Tajima et al., 2004) (Figure 3C). A potential target of type-B ARRs in promoting cell cycle reentry is CYCD3, since its expression is upregulated within 1 h after cytokinin treatment and overexpression of CYCD3 enhances callus formation in the absence of exogenous cytokinin (Riou-Khamlichi et al., 1999). Consistently, loss of CYCD3, together with its close homologs CYCD3;2 and CYCD3;3, leads to a reduced cytokinin response, strongly suggesting that CYCD3s function as a downstream effector of cytokinin signaling (Dewitte et al., 2007).
The AP2/ERF transcription factors ENHANCED SHOOT REGENERATION (ESR; also known as DORNROSCHEIN [DRN]), ESR1, and ESR2, are other candidates that may function in cytokinin-mediated callus formation, since overexpression of ESR1 or ESR2 induces callus without exogenous plant hormones (Banno et al., 2001; Ikeda et al., 2006) (Figures 3D and 4B). Similar callus induction is present in the activation tagging line BOLITA (BOL), the same locus as ESR2 (Marsh-Martinez et al., 2006). The ESR proteins may link cytokinin signaling to cell cycle control since ESR2 directly activates the expression of CYCD1;1 and the DOF transcription factor OBF BINDING PROTEIN1 (OBP1) (Okamuro et al., 1997). The OBP1 gene is known to promote cell cycle reentry by shortening the duration of the G1 phase (Skirycz et al., 2008). Overexpression of OBP1 causes upregulation of many cell cycle–related genes and OBP1 directly binds the promoter sequence of CYCD3;3 and the S phase–specific transcription factor DOF2;3 (Skirycz et al., 2008). Future experiments are needed to validate whether these ESR-mediated pathways underlie cell cycle reactivation during callus induction, but these findings support the view that cell cycle reentry is governed by multiple layers of transcriptional regulations to orchestrate the expression of several cell cycle genes.

**Callus Induction by Wounding**

Mechanical damage has long been recognized as a common stimulus of callus induction, but the molecular mechanisms underlying this response are poorly understood. An AP2/ERF transcription factor, WOUND INDUCED DEDIFFERENTIATION1 (WIND1), and its close homologs WIND2, WIND3, and WIND4 are the central regulators of this response recently identified in Arabidopsis (Iwase et al., 2011a, 2011b) (Figure 4C). WIND1, initially called RAP2.4 (Okamuro et al., 1997), was described as one of the wound-inducible genes (Delessert et al., 2004), and expression of all four WIND genes is strongly upregulated within a few hours of wounding (Iwase et al., 2011a). Neither the single loss-of-function mutants in WIND1-4 nor their quadruple mutants affect callus induction at the wound site, but dominant repression of WIND1, effected by expressing chimeric WIND1-SRDX (SUPERMAN repression domain) proteins, results in reduced callus formation in wounded hypocotyls (Iwase et al., 2011a). Therefore, WIND proteins appear to cooperate with other functionally redundant factors to mediate callus formation upon wounding.

The ectopic overexpression of individual WIND genes is sufficient to induce callus (Iwase et al., 2011a) (Figure 3E), and these WIND-induced calli can be subcultured on phytohormone-free media while maintaining their proliferative competence (Iwase et al., 2011b). Chemically induced overexpression of WIND1 also leads to the production of somatic embryos (Figure 3F), and when transferred to noninducible media, they regenerate whole plants. These observations suggest that excess levels of WIND1 proteins are sufficient to induce cell dedifferentiation and that WIND1-expressing cells are totipotent. Th-WIND1-L is an ortholog of Arabidopsis WIND1 in salt cress (Thellungiella halophila), a close relative of Arabidopsis (Zhou et al., 2012). Th-WIND1-L expression is also wound inducible, and Arabidopsis plants overexpressing Th-WIND1-L display callus formation without exogenous plant hormones (Zhou et al., 2012), suggesting that the function of WIND proteins in the wound-induced callus formation is conserved across plant species.

So how do WIND proteins promote callus induction? Current data suggest that WIND proteins act through a cytokinin-mediated pathway since WIND1-induced callus formation is strongly repressed in arr1 arr12 double mutants defective in type-B ARR-mediated cytokinin signaling (Figure 4C). Consistently, wounding upregulates type-B ARR-mediated cytokinin response, as visualized by the expression of green fluorescent protein (GFP) under a two-component-output sensor promoter, and this response is dependent on WIND1 (Iwase et al., 2011a). How WIND proteins activate cytokinin signaling is elusive, but identification of transcriptional downstream targets of WIND should unveil these molecular links in the future.

Given that wound-induced callus formation is not abolished completely in WIND1-SRDX plants, it is likely that additional factors participate in this response in parallel to WIND proteins. The pressing question is how wound signals promote cell cycle reentry through the WIND-dependent and/or -independent pathways, but at present, most of these regulatory cascades remain unknown. The expression of the CDKA;1 gene is upregulated within 30 min at the wound site in Arabidopsis leaves (Hemerly et al., 1993), but functional relevance of this upregulation has not been fully investigated.

In the moss Physcomitrella patens, wounding induces reprogramming of gametophyte leaf cells into chloronema apical cells. This response is an elegant example of cell dedifferentiation involving both cell cycle reactivation and acquisition of a new cell fate. A recent study by Ishikawa et al. (2011) demonstrated that the wound signal promotes the expression of CYCD;1 at the wound site and through its binding to CDKA, upregulates CDKA activity. The expression of dominant-negative CDKA;1 or treatment with roscovitine, a CDK inhibitor, blocks both cell cycle reentry and cell fate acquisition, highlighting the pivotal roles of the CYCD;1-CDKA complex in wound-induced reprogramming.

Wounding also induces tissue or organ regeneration and the underlying molecular mechanisms are beginning to be understood in Arabidopsis. Although these processes do not involve extensive overproliferation, they appear to involve dedifferentiation of somatic cells. For instance, excision of the root tip initiates rapid regeneration of lost tip. The first transcriptional change indicative of cell fate reestablishment is detectable within several hours after injury and functional root tips are restored within 24 h (Sena et al., 2009). Remaining meristematic cells participate in the regeneration, suggesting that meristematic cells outside the stem cell niche still possess the competence to dedifferentiate upon wounding. Strikingly, these regeneration processes do not require the activity of a stem cell niche since Arabidopsis mutants defective in stem cell maintenance are not impaired in the formation of new root tips (Sena et al., 2009). Another case of regeneration is found after the
incision of Arabidopsis inflorescence stems in which fully elongated pith and cortex cells reinitiate cell proliferation to heal the wound site (Asahina et al., 2011). Auxin is the central player mediating this response since chemical or genetic perturbation of polar auxin transport strongly impedes the stem regeneration. Auxin accumulates at the upper region of the cut stem, which in turn induces the expression of Arabidopsis NAC DOMAIN CONTAINING PROTEIN71 (ANAC071), while auxin is depleted at the lower region of the cut stem, resulting in the increased expression of an AP2/ERF transcription factor RAP2.6L. Dominant suppressor of ANAC071 or RAP2.6L abolishes wound-induced cell proliferation, strongly suggesting that they are essential regulators in the regeneration process (Figure 4C). The next important questions are why and how wound promotes different responses in different contexts. Elucidating how wound signals are perceived and transduced in each event should provide some important clues to answer this question.

Callus Induction by the Reacquisition of Embryonic or Meristematic Fate

Numerous studies in recent years have shown that ectopic overexpression of embryonic regulators or meristematic regulators induces callus formation in various plant species (Figure 4D). These findings illustrate that excess activation of a relatively undifferentiated cell fate is sufficient to drive unorganized cell proliferation. A CCAAT-box binding transcription factor LEAFY COTYLEDON1 (LEC1), a B3 domain transcription factor LEC2, and a MADS box transcription factor AGAMOUS-LIKE15 (AGL15) function as a transcriptional activator during embryogenesis. When either of these transcription factors is ectopically expressed in Arabidopsis, the resulting plants produce embryonic callus on phytohormone-free medium (Lotan et al., 1998; Stone et al., 2001; Harding et al., 2003; Gaj et al., 2005; Umehara et al., 2007; Thakare et al., 2008) (Figure 3G). An AP2/ERF transcription factor BABY BOOM (BBM) was initially identified in Brassica napus, and Brn-BBM is preferentially expressed during embryogenesis and seed development (Boutilier et al., 2002). Interestingly, overexpression of Brn-BBM induces embryonic callus in both Brassica and Arabidopsis without exogenous plant hormones (Boutilier et al., 2002). The transient overexpression system of Brn-BBM has been applied successfully in several crop and tree species to increase the efficiency of callus induction and consequently promote redifferentiation into individual plants (Srinivasan et al., 2007; Deng et al., 2009; Heidmann et al., 2011). It is also known that the soybean (Glycine max) BBM induces embryonic callus in Arabidopsis seedlings (El Ouakfaoui et al., 2010), suggesting that the function of BBM in promoting embryogenesis or embryonic callus formation might be conserved across dicotyledonous plants. These properties might be shared among related AP2/ERF proteins since ectopic expression of a close homolog of BBM in Arabidopsis, EMBRYOMAKER (EMK), also known as AINTEGUMENTA-LIKE5 (AIL5) or PLETHORA5 (PLT5), also facilitates similar embryonic callus development (Tsuwamoto et al., 2010).

The RKD (RWP-RK domain-containing) proteins are another class of putative transcription factors implicated in female gametogenesis and early embryogenesis. RKD1 and RKD2 are expressed preferentially expressed in the egg cell, and their ectopic overexpression in Arabidopsis induces callus without exogenous plant hormones (Köszegi et al., 2011) (Figure 4D). Microarray experiments suggested that the gene expression profile of RKD2-induced callus is closer to that of egg cells than to auxin-induced callus (Köszegi et al., 2011), implying that RKD2 overexpression drives callus formation by activating the egg cell fate. RKD4 is expressed in early embryos and chemically induced activation of RKD4 promotes transcription of early embryo-specific genes and unorganized cell proliferation in Arabidopsis roots (Waki et al., 2011) (Figures 3H and 4D).

The plant meristem is the ultimate source of all tissues in the plant body, and these generative activities are supported by a pool of stem cells residing within the meristem. Thus, it is not surprising that strong activation of these meristematic activities leads to ectopic callus induction. The homeodomain-containing transcription factor WUSCHEL (WUS) is expressed in the stem cell organizing center of shoot meristems and is required to maintain stem cells in a relatively undifferentiated state (Laux et al., 1996; Mayer et al., 1998). WUS is also strongly expressed in several callus lines (Iwase et al., 2011a), and Arabidopsis plants overexpressing WUS generate callus as well as somatic embryos (Zuo et al., 2002) (Figures 3I and 4D).

RNA Processing and Protein Translation during Callus Formation

The process of callus induction involves massive changes in gene expression to alter the level of cell differentiation and dedifferentiation. We have so far described various regulators responsible for these transcriptional modifications, but several lines of evidence suggest that failures in accurate RNA production and/or processing constrain callus generation. The SHOOT REDERIFICATION DEFECTIVE2 (SRD2) gene encodes a nuclear protein that has sequence similarity to the human SNAPP50, a protein required for the transcription of small nuclear RNA (snRNA). The srd2 mutants are incapable of transcribing snRNA at the restrictive temperature, and, strikingly, these defects disturb CIM-induced callus formation from hypocotyl explants (Ozawa et al., 1998; Ohtani and Sugiyama, 2005). The snRNA is thought to function in RNA splicing as a component of spliceosome (Burge et al., 1999, and references therein); thus, SRD2-mediated production of snRNA appears to be essential for pre-mRNA splicing during CIM-induced callus formation (Ohtani and Sugiyama, 2005).

Koukalova et al. (2005) detected an elevation of rRNA transcription during hormone-induced callus formation in tobacco leaf explants. Similarly, Ohbayashi et al. (2011) reported an accumulation of the rRNA precursors during CIM-induced callus initiation from Arabidopsis hypocotyls, inferring an involvement of active rRNA biogenesis in callus induction. In agreement with this, a mutation in ROOT INITIATION DEFECTIVE2 (RID2), a nuclear-localized methyltransferase-like protein, impedes CIM-induced callus formation at the restrictive temperature, and these phenotypes are accompanied by aberrant accumulation of various pre-rRNA intermediates (Konishi and Sugiyama, 2003; Ohbayashi et al., 2011).
Both SRD2 and RID2 are expressed in meristematic tissues, and their transcription is induced after incubation on CIM, indicating that their activities are tightly linked with high proliferative capacities of cells (Ohtani and Sugiyama, 2005; Obayashi et al., 2011). These posttranscriptional processes might not be the initial trigger of callus induction, and they are more likely to produce new sets of proteins required for callus formation. Previous proteomic analyses have indeed uncovered dynamic alterations in the nuclear protein profile of Arabidopsis cotyledons undergoing callus induction (Chittetti and Peng, 2007; Chittetti et al., 2008).

MOLECULAR BASIS OF CALLUS REPRESSION

Maintaining the correct body structure and tissue organization is a prerequisite for the full growth and functioning of plants; thus, plant cells must be able to prevent unscheduled overproliferation. In this section, we will discuss how callus induction is repressed by both genetic and epigenetic mechanisms.

Cell Wall Integrity

Orderly deposition of structural cell wall materials, such as cellulose, hemicellulose, and pectin, is critical for establishing and/or maintaining the cellular differentiation status (Figure 5A). Loss-of-function mutations in cell wall production often lead to callus formation. For example, a mutant of GLUCURONYLTRANSFERASE1 (GUT1) in Nicotiana plumbaginifolia, called nonorganogenic callus with loosely attached cells (nolac-H18), develop callus on the shoot apex (Iwai et al., 2002). The GUT1 protein is required for the biosynthesis of pectin as it transfers glucuronic acid to rhamnogalacturonan II, one of the most prevalent forms of pectin in plants. The glucuronic acid level of rhamnogalacturonan II is strongly reduced in the nolac-H18 mutant, thus disrupting the matrix organization in the primary cell wall (Iwai et al., 2002). Arabidopsis loss-of-function mutants TUMOROUS SHOOT DEVELOPMENT1 (tsd1) and tsd2 develop a disorganized mass of cells that grow indefinitely on hormone-free medium (Frank et al., 2002). TSD1, previously identified as KORRIGAN1 (KOR1) and RADIAL SWELLING2 (RSW2), encodes a membrane-bound endo-1,4-β-D-glucanase involved in the biosynthesis of cellulose (Nicol et al., 1998; Zuo et al., 2000; Lane et al., 2001; Krupková and Schmülling, 2009; Figure 3J). The tsd1/kor1/rsw2 mutants are impaired in cellulose production, and these defects are also accompanied by marked changes in the pectin composition, together resulting in distorted cellular organization of shoots and roots (Nicol et al., 1998; His et al., 2001). TSD2, also known as QUASIMODO2 (QUA2) and OVERSENSITIVE TO SUGAR1 (OSU1), encodes a putative Golgi-localized methyltransferase (Mouille et al., 2007; Ralet et al., 2008; Gao et al., 2008). How TSD2/QUA2/OSU1 affects cell wall biosynthesis is not known, but tsd2/qua2/osu1 mutants show 50% reduction in the level of homogalacturonan, another major component of pectin, leading to severe defects in cell adhesion (Krupková et al., 2007; Mouille et al., 2007; Ralet et al., 2008). The overproliferation phenotypes of these cell wall mutants presumably are an indirect consequence of disrupted intercellular communication. Based on various marker expression analyses, the callus-forming phenotype of tsd1/kor1/rsw2 appears to associate with ectopic acquisition of shoot meristem identity and an enhanced cytokinin response (Krupková and Schmülling, 2009) (Figure 5A). For instance, the expression of SHOOTMERISTEMLESS and CLAVATA3 normally is restricted to the shoot apical meristem in wild-type seedlings, but both genes are ectopically expressed in tsd1/kor1/rsw2 callus (Krupková...
and Schnülling, 2009). Furthermore, cytokinin signaling is strongly elevated in tsd1/kor1/rsw2 mutants, and overexpression of CYTOKININ OXIDASE1, a gene encoding a cytokinin-degrading enzyme, partially rescues the overproliferation phenotype in tsd1/kor1/rsw2 mutants (Krupkova and Schnülling, 2009). Together, these results suggest that the correct deposition of cell wall materials is critical for coordinating tissue differentiation and in preventing overproliferation of somatic cells.

Epigenetic Regulation

Epigenetic regulators affect gene expression by chromatin modification, including DNA methylation and histone modification. Global chromatin status regulated by these epigenetic regulators is conceived to play central roles in the control of cell differentiation and de-differentiation (reviewed in Gaspar-Maia et al., 2011; Grafi et al., 2011). In mammals, cells with determined fate generally have a closed chromatin state with relatively stable gene expression profile, while pluripotent cells have an open state that is ready for dynamic change in gene expression (Gaspar-Maia et al., 2011). Whether a similar regulatory system operates in plants is not established, but several cytological studies suggest that the chromatin state in plant nucleus is also modified depending on the status of cellular differentiation (Zhao et al., 2001; Verdeil et al., 2007).

Polycomb Repressive Complex1 (PRC1) and PRC2 are evolutionarily conserved protein complexes involved in histone modification. In animals, PRC2 trimethylates histone H3 on Lys-27 (H3K27me3), a mark of transcriptionally silent chromatin, which in turn recruits PRC1 to mono-ubiquitinate histone H2A on Lys-119 (H2AK119ub), a mark that stabilizes this silencing effect. The molecular function of PRCs in depositing repressive histone marks appears to be conserved in plants, but their mode of action might be slightly different since at least in some cases in Arabidopsis, H2AK119ub initiates repression of target gene expression and H3K27me3 maintains their repressive status (Yang et al., 2013). The PRCs were first identified from loss-of-function mutants in Drosophila melanogaster with ectopic organ formation; accordingly, they primarily function in the maintenance of various cell fates during developmental processes (reviewed in Ringrose and Paro, 2004). A considerable body of evidence suggests that plant PRCs are required for the stable repression of embryonic and meristic program in differentiating organs (Figure 5B). Most of the PRC2 components are encoded by partially redundant genes in Arabidopsis, and double mutants of these homologs, for example, CURLY LEAF (CLF) and SWINGER (SWN), or VERNALIZATION2 (VRN2) and EMBRYONIC FLOWER2 (EMF2), exhibit spontaneous callus formation similar to wild type after germination (Chanvitattana et al., 2004; Schubert et al., 2005, 2008). Similar callus formation is also reported for a mutant of FERTILIZATION-INDEPENDENT ENDOSPERM (FIE), another component of PRC2 encoded by a single gene in Arabidopsis (Bouyer et al., 2011). Whether plants possess PRC1 has long been questioned, but recent studies have identified At-BMI1A and At-BMI1B, homologs of the Ring finger proteins in mammalian PRC1, in Arabidopsis (Sanchez-Pulido et al., 2008). Similar to the mutations in PRC2, the At-bmi1a-1 bmi1b double mutants are unable to continue and/or maintain differentiation, and they form callus at an early stage of postembryonic development (Bratzel et al., 2010) (Figure 3L). These callus phenotypes in PRC mutants are accompanied by ectopic expression of embryonic regulators, such as LEC1, LEC2, AGL15, and BBM, as well as several meristic regulators, such as WUS and WUSHEL RELATED HOMEBOX5 (WOX5) (Bratzel et al., 2010; Bouyer et al., 2011), most of which, as discussed above, promote callus generation when overexpressed. In addition, it has been recently shown that many of these genes have H3K27me3 and H2AK119ub marks, strongly suggesting that they are directly targeted by PRC1 and PRC2 to repress callus formation (Bratzel et al., 2010; Bouyer et al., 2011; Yang et al., 2013).

The PICKLE (PKL) protein, a Chromodomain-Helicase-DNA binding3 (CHD3) and CHD4-like chromatin remodeling factor, may also play a central role in the repression of unscheduled overproliferation since the pkl mutants develop callus soon after germination (Ogas et al., 1997, 1999) (Figure 5B). The CHD3/CHD4 class of chromatin remodelers acts as histone deacetylases in animals (Hollender and Liu, 2008). A recent study identified another allele of pkl mutants called cytokinin-hyper-sensitive2, which displays an elevated response to exogenous cytokinin in an in vitro callus induction assay (Furuta et al., 2011). This phenotype can be partially phenocopied by the application of trichostatin A, an inhibitor of histone deacetylases, suggesting that PKL functions in histone deacetylation (Furuta et al., 2011). In addition, PKL appears to participate in the deposition of H3K27me3 since PKL is present at the LEC1 and LEC2 loci in young seedlings and their H3K27me3 levels are reduced in pkl mutants, resulting in their derepression and, hence, callus induction (Zhang et al., 2008, 2012) (Figure 5B).

Several recent studies have shown that some components of the chromatin modifiers directly interact with transcription factors implicated in embryogenesis and, together, they modify chromatin status to regulate the expression of specific target genes (Figure 5B). For example, the At-BMI1 protein in PRC1 interacts with a B3 domain transcription factor VP1/AB13-LIKE1 (VAL; also known as HIGH-LEVEL EXPRESSION OF SUGAR-INDUCIBLE GENE2 [HSI2]) to repress the expression of LEC1 and LEC2 through H2AK119ub (Yang et al., 2013). In addition, a close homolog of VAL/HSI2, VAL2/HSI2-LIKE1 (HSL1), acts together with HISTONE DEACETYLASE19 (HDA19) to repress LEC1 and LEC2 expression by deacetylation of histone H3 (H3ac) and H4 (H4ac) (Zhou et al., 2013). An interesting hypothesis that may explain these interactions is that the transiently expressed transcription factors recruit epigenetic regulators to specific targets and modify their gene expression in a spatially and temporally controlled manner. A previous study has shown that VAL1/HSI2 and VAL2/HSL1 act redundantly in repressing these embryonic genes and thereby callus induction (Tsukagoshi et al., 2007), suggesting that callus formation is suppressed by both H2AK119ub and H3/H4Ac in postembryonic tissues.

CONCLUSIONS AND FUTURE PERSPECTIVES

Plants develop callus or other tumors after exposure to various harsh growth conditions. This is obviously a big commitment for plants since they have to give up their fully established body plans and start a new developmental program once again. What we have learnt so far from recent studies is that many of these
naturally occurring callus are formed through the modulation of plant hormone signaling, in particular, of auxin and cytokinin. We now know that several key regulators of these hormone signaling pathways (e.g., ARFs and ARRs) function during callus induction, but more work is needed to decipher how they promote the reacquisition of cell proliferative competence. It is also becoming clear that the formation of some calli uses intrinsic developmental programs, such as embryogenesis and meristem formation. These programs are spatially and temporally restricted under normal growth conditions but appear to get ectopically activated after experiencing certain environmental challenges. It is likely that these hormonal and developmental pathways are interconnected at multiple levels, and further dissection of these highly intersecting molecular networks offers one of the major challenges in future studies. We are beginning to uncover novel regulators, such as WiND proteins, that translate stress signals into the control of cell differentiation. Elucidating their upstream and downstream regulatory cascades in model plants will be an important next step to unveil the complete regulatory mechanisms underlying callus formation. Exploring the molecular basis of pathogen-induced tumorigenesis is another exciting area of central importance. Different types of pathogens (e.g., viruses, bacteria, fungi, and insects) hijack the plant developmental program probably using their own unique strategies. Rapidly advancing technology of next-generation sequencing now allows us to investigate the transcriptional changes in nonmodel plants so we can compare various forms of cellular dedifferentiation processes in different species at the molecular level.

We are also beginning to understand how embryonic and meristematic programs are epigenetically repressed. In mammals, key transcription factors conferring pluripotency (Oct4, Sox2, Nanog, and c-Myc) are repressed by multiple and distinct epigenetic mechanisms, such as DNA methylation, H3K9me3, or H3K27me3, thus ensuring the robust maintenance of cellular differentiation program (Hawkins et al., 2010). Currently available data suggest that plants may have less redundant mechanisms for epigenetic repression, and it will be interesting to explore whether these properties underlie the higher dedifferentiation capacities of plant cells.

We should note that studying callus has numerous important implications in other areas of biology as it addresses questions of, for example, how multicellular organisms perceive and transduce endogenous and environmental signals and how they induce or maintain cell differentiation/dedifferentiation. Given that the classical hormone-based technologies of plant propagation or transformation are applicable only to limited species or accessions, insights gained from basic callus research also have promising downstream application potentials. Once we fully understand how genetic and epigenetic mechanisms cooperate to balance cell differentiation and dedifferentiation, this knowledge should help us design more sophisticated and more specific molecular tools to systematically manipulate organ regeneration.

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