The Plant Cell, Vol. 25: 3159–3173, September 2013, www.plantcell.org

REVIEW

Plant Callus: Mechanisms of Induction and Repression[OPEN]

Momoko Ikeuchi, Keiko Sugimoto, and Akira Iwase[1]

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

[1] Address correspondence to akira.iwase@riken.jp.
[OPEN] Articles can be viewed online without a subscription.
www.plantcell.org/cgi/doi/10.1105/tpc.113.116053

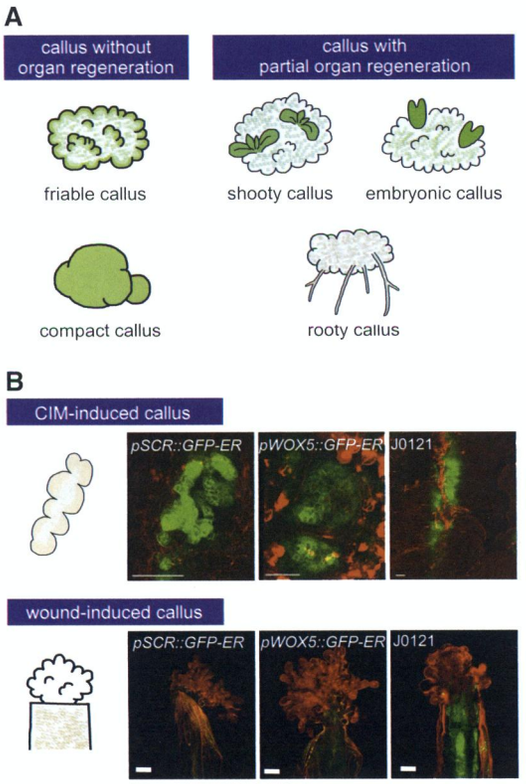

Figure 1. Schematic Illustration of Various Types of Plant Callus.

(A) Calli without any obvious organ regeneration are typically called friable or compact callus depending on their tissue characteristics. Calli with some degrees of organ regeneration are often called rooty, shooty, or embryonic callus depending on the organs they form.

(B) Comparison between callus generated on auxin- and cytokinin-containing CIM and callus generated at the wound site. While root meristem markers (*pSCR:GFP-ER* and *pWOX5:GFP-ER*) and a root pericycle marker (J0121) are expressed in CIM-induced callus (Sugimoto et al., 2010), none of these markers are expressed in wound-induced callus (Iwase et al. 2011a). Scale bars = 50 μm. (*Microscopy images in **[B]** are reprinted from Sugimoto et al. [2010], Figure 3E [left], 3E [center], and 3B [right] and from Iwase et al. [2011a], Supplemental Figure 1H [right and center] with permission from Cell Press.*)

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 formation4* 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 monooxygenase, 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).

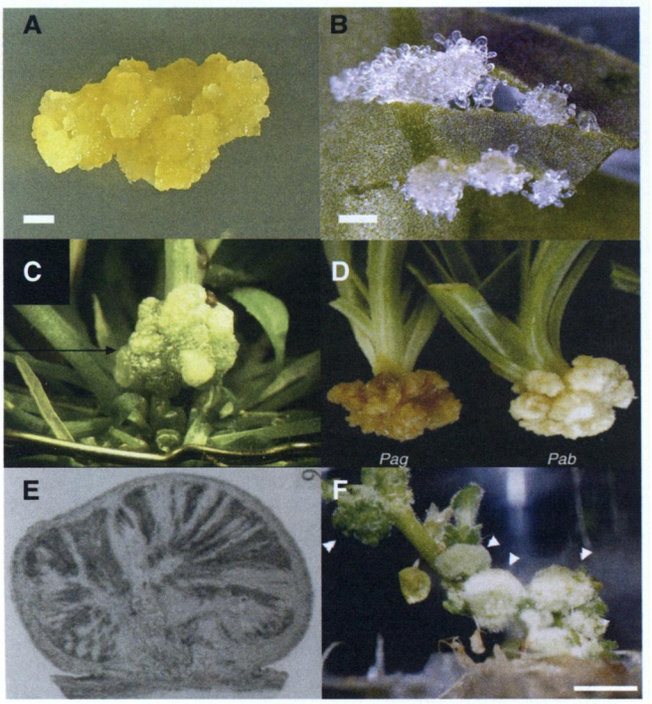

Figure 2. Callus Formation in Vitro and in Nature.

(A) Callus formed under in vitro culture condition. The *Arabidopsis* seedling was cultured on CIM from germination and the photograph was taken after 30 d.

(B) Callus induced at the wound site. The *Arabidopsis* leaf was partly cut by fine scissors, and the photograph was taken after 6 d.

(C) Tumors induced by bacterial infection. The wounded *Arabidopsis* inflorescence stalk was inoculated with the gram-negative bacteria *Agrobacterium* strain C58. The black arrow indicates an unorganized cell mass, called crown gall, developing after 30 d from inoculation (Eckardt, 2006).

(D) Two-week-old galls on gypsophila cuttings inoculated with *P. agglomerans* pv *gypsophilae* (*Pag*) or *P. agglomerans* pv *betae* (*Pab*) (Barash and Manulis-Sasson, 2007).

(E) Longitudinal section of a gall that developed by WTVs on the shoot of sweet clover (Lee, 1955).

(F) Genetic tumors induced by interspecific crosses between *Nicotiana glauca* and *Nicotiana langsdorffii*. Arrowheads indicate callus growing on the F1 hybrid plant (Udagawa et al. 2004).

Bars = 1 mm in **(A)** and **(F)** and 500 μm in **(B)**. (*Image in **[C]** reprinted from Eckardt [2006], Figure 1B courtesy of Rosalia Deeken; **[D]** is reprinted from Barash and Manulis-Sasson [2007], Figure 1 with permission from Elsevier; **[E]** is reprinted from Lee [1955], Figure 9 with permission from Botanical Society of America; **[F]** is reprinted from Udagawa et al. [2004], Figure 4A with permission from Oxford University Press.*)

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*

Locus	Common Name	Protein Family	Predicted Function	References
AT2G42430[a]	*LBD16*	LOB-domain transcription factor (TF)	Auxin response/lateral root formation	Fan et al. (2012)
AT2G42440[a]	*LBD17*	LOB-domain TF	Auxin response	Fan et al. (2012)
AT2G45420[a]	*LBD18*	LOB-domain TF	Auxin response/lateral root formation	Fan et al. (2012)
AT3G58190[a]	*LBD29*	LOB-domain TF	Auxin response/lateral root formation	Fan et al. (2012)
AT3G16857[a]	*ARR1*	GARP TF	Cytokinin response	Sakai et al. (2001)
AT5G07210[a]	*ARR21*	GARP TF	Cytokinin response	Tajima et al. (2004)
AT1G12980[a]	*ESR1/DRN*	AP2/ERF TF	Cytokinin response/shoot regeneration	Banno et al. (2001)
AT1G24590[a]	*ESR2/DRNL/BOL*	AP2/ERF TF	Cytokinin response/shoot regeneration	Ikeda et al. (2006); Marsch-Martinez et al. (2006)
AT1G78080[a]	*WIND1/RAP2.4b*	AP2/ERF TF	Wound-induced cell dedifferentiation	Iwase et al. (2011a, 2011b)
AT1G22190[a]	*WIND2/RAP2.4d*	AP2/ERF TF	Wound-induced cell dedifferentiation	Iwase et al. (2011a, 2011b)
AT1G36060[a]	*WIND3/RAP2.4a*	AP2/ERF TF	Wound-induced cell dedifferentiation	Iwase et al. (2011a, 2011b)
AT5G65130[a]	*WIND4*	AP2/ERF TF	Wound-induced cell dedifferentiation	Iwase et al. (2011a, 2011b)
AT1G21970[a]	*LEC1*	CCAAT-box binding TF	Embryogenesis	Lotan et al. (1998)
AT1G28300[a]	*LEC2*	B3 domain TF	Embryogenesis	Stone et al. (2001)
AT5G13790[a]	*AGL15*	MADS box TF	Embryogenesis	Harding et al. (2003)
AT5G17430[a]	*BBM*	AP2/ERF TF	Embryogenesis	Boutilier et al. (2002)
AT5G57390[a]	*EMK/AIL5/PLT5*	AP2/ERF TF	Embryogenesis	Tsuwamoto et al. (2010)
AT1G18790[a]	*RKD1*	RWP-RK domain TF	Gametogenesis	Kőszegi et al. (2011)
AT1G74480[a]	*RKD2*	RWP-RK domain TF	Gametogenesis	Kőszegi et al. (2011)
AT5G53040[a]	*RKD4*	RWP-RK domain TF	Embryogenesis	Waki et al. (2011)
AT2G17950[a]	*WUS*	Homeodomain TF	Stem cell maintenance	Zuo et al. (2002)
AT3G50360[b]	*KRP2*	CDK inhibitor	Negative regulation of cell proliferation	Anzola et al. (2010)
AT5G48820[b]	*KRP3*	CDK inhibitor	Negative regulation of cell proliferation	Anzola et al. (2010)
AT1G49620[b]	*KRP7*	CDK inhibitor	Negative regulation of cell proliferation	Anzola et al. (2010)
AT5G49720[b]	*TSD1/KOR1/RSW2*	Endo-1,4-β-D-glucanase	Cellulose biosynthesis	Frank et al. (2002); Krupková and Schmülling (2009)
AT1G78240[b]	*TSD2/QUA2/OSU1*	*S*-adenosyl-L-Met–dependent methyltransferase	Pectin biosynthesis (?)	Frank et al. (2002); Krupková et al. (2007)
AT2G23380[b]	*CLF*	PRC2	Histone H3 Lys-27 trimethylation	Chanvivattana et al. (2004)
AT4G02020[b]	*SWN*	PRC2	Histone H3 Lys-27 trimethylation	Chanvivattana et al. (2004)
AT4G16845[b]	*VRN2*	PRC2	Histone H3 Lys-27 trimethylation	Chanvivattana et al. (2004); Schubert et al. (2005)
AT5G51230[b]	*EMF2*	PRC2	Histone H3 Lys-27 trimethylation	Chanvivattana et al. (2004); Schubert et al. (2005)
AT3G20740[b]	*FIE*	PRC2	Histone H3 Lys-27 trimethylation	Bouyer et al. (2011)
AT2G30580[b]	At *BMI1A*	PRC1	Histone H2A Lys-119 ubiquitination	Bratzel et al. (2010)
AT1G06770[b]	At *BMI1B*	PRC1	Histone H2A Lys-119 ubiquitination	Bratzel et al. (2010)
AT2G25170[b]	*PKL*	CHD3/4-like chromatin remodeling factor	Histone H3 Lys-27 trimethylation and histone deacetylation (?)	Ogas et al. (1997, 1999)
AT2G30470[b]	*VAL1/HSI2*	B3 domain TF	Termination of embryogenesis	Tsukagoshi et al. (2007)
AT4G32010[b]	*VAL2/HSL1*	B3 domain TF	Termination of embryogenesis	Tsukagoshi et al. (2007)

[a]Genes that promote callus formation upon overexpression.
[b]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,

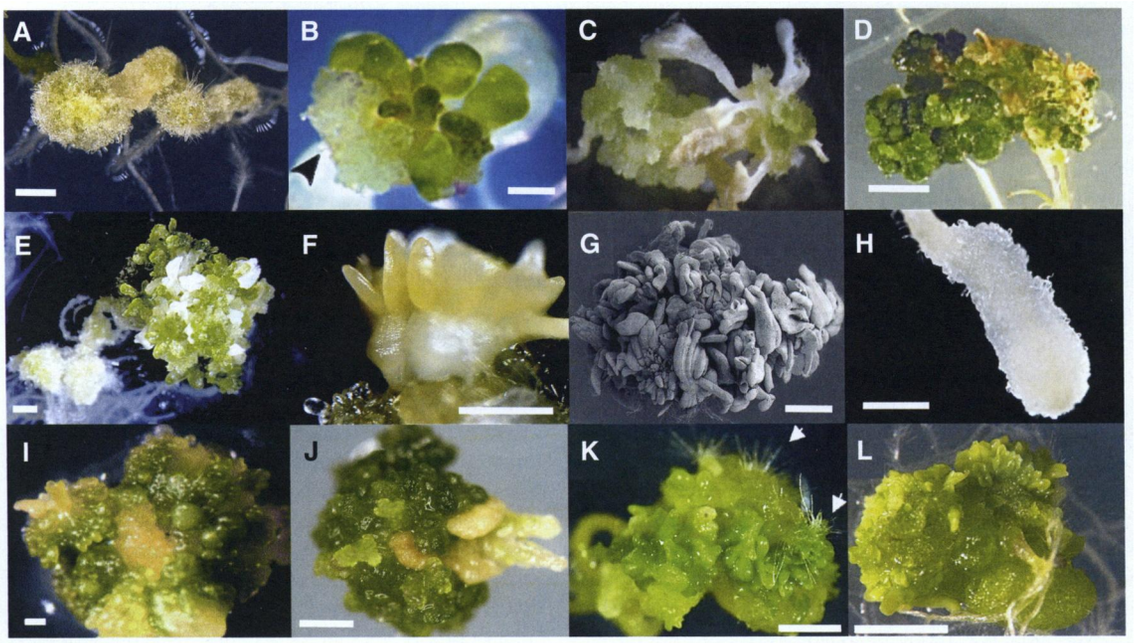

Figure 3. Gain-of-Function and Loss-of-Function Mutants Exhibiting Ectopic Callus Formation in *Arabidopsis*.

(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 calls growing on the shoot, hypocotyl, and root of *WIND1*-overexpressing plants (Iwase et al., 2011a).
(F) Somatic embryos generated on *WIND1*-overexpressing callus.
(G) Embryonic callus 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.*)

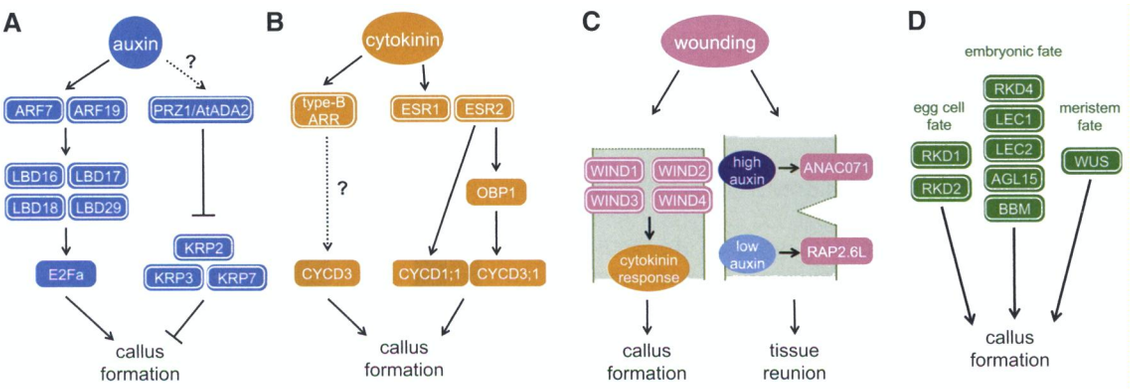

Figure 4. Molecular Mechanisms of Callus Induction.

(A) Auxin-induced callus formation. Auxin signaling is transduced via ARF transcription factors, especially ARF7 and ARF19, to activate the expression of LBD family transcription factors, LBD16, LBD17, LBD18, and LBD29. These LBDs in turn induce E2Fa, a transcription factor that plays a central role in cell cycle reentry. The PRZ1/AtADA2 protein mediates auxin-dependent repression of CDK inhibitors, KRP2, KRP3, and KRP7. How auxin modulates the expression and/or activity of PRZ1/AtADA2 is currently unknown.

(B) Cytokinin-induced callus formation. Cytokinin signaling is transduced via two-component regulatory pathway to activate the type-B ARR transcription factors. The expression of CYCD3;1 is sharply upregulated by cytokinin, but whether it is directly activated by type-B ARR is not known. The AP2/ERF transcription factor ESR1 is also upregulated by cytokinin. ESR1 and its functionally redundant homolog ESR2 might mediate cell cycle reactivation since ESR2 induces the expression of CYCD1;1 as well as a DOF binding transcription factor OBP1. OBP1 is thought to promote the cell cycle progression by inducing expression of CYCD3;3 and several other cell cycle regulators.

(C) Wound-induced callus formation. Complete excision of the *Arabidopsis* hypocotyls induces the expression of *WIND1*, *WIND2*, *WIND3*, and *WIND4* genes at the wound site, which in turn upregulates the cytokinin response to promote callus formation. When *Arabidopsis* stems are half-cut, auxin transported from the shoot apex accumulates at the upper end of the wound site, which then induces the expression of *ANAC071* gene. Auxin is depleted from the lower end, resulting in the induction of the *RAP2.6L* gene. Both of these responses are required for the local activation of cell proliferation to heal the gap at the wound site. Dotted lines indicate the wound site.

(D) Callus formation by the reacquisition of embryonic or meristematic fate. Overexpression of each of the master regulators in the egg cell fate (RKD1 and RKD2), embryonic fate (RKD4, LEC1, LEC2, AGL15, and BBM), or meristem fate (WUS) is sufficient to induce callus formation. Proteins with confirmed function in callus formation are highlighted with white circles, while those inferred in callus formation based on indirect evidence are unmarked.

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 PROPORZ1 (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 phosphorelay 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;1*, 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 DORNRÖSCHEN [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* (Marsch-Martinez et al., 2006). The ESR proteins are implicated in the cytokinin signaling pathway because ESR-overexpressing plants show elevated responses to cytokinin and they rescue the regeneration defects of cytokinin receptor mutants *cytokinin response1/Arabidopsis histidine kinase4* (Banno et al., 2001; Ikeda 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*) (Ikeda et al., 2006). 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 halophile*), 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 suppression 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 wounding 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 Bn-*BBM* is preferentially expressed during embryogenesis and seed development (Boutilier et al., 2002). Interestingly, overexpression of Bn-*BBM* induces embryonic callus in both *Brassica* and *Arabidopsis* without exogenous plant hormones (Boutilier et al., 2002). The transient overexpression system of Bn-*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 (Kőszegi 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 (Kőszegi 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 REDIFFERENTIATION DEFECTIVE2* (*SRD2*) gene encodes a nuclear protein that has sequence similarity to the human SNAP50, 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; Ohbayashi 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 (Chitteti and Peng, 2007; Chitteti 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

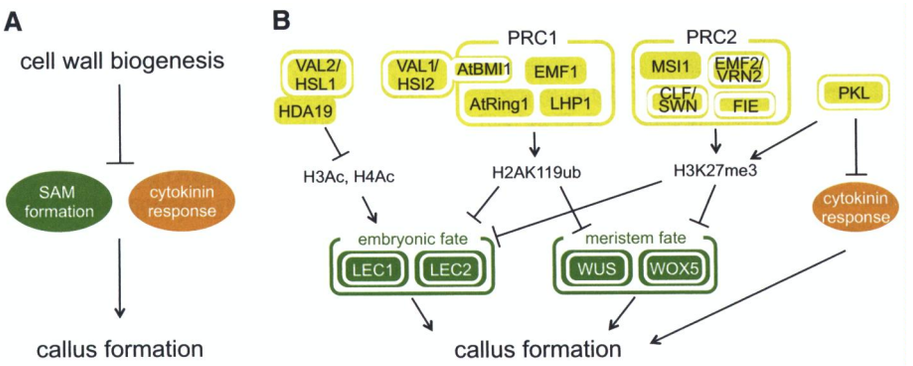

Figure 5. Molecular Mechanisms of Callus Repression.

(A) Orderly deposition of cell wall polysaccharides prevents ectopic callus formation. Defects in cell wall biosynthetic enzymes (e.g., *nolac-H18* in tobacco and *tsd1* and *tsd2* in *Arabidopsis*) result in the ectopic expression of shoot apical meristem (SAM) genes and increased cytokinin response, leading to callus induction as an indirect downstream consequence.

(B) Ectopic callus formation is repressed by multiple epigenetic mechanisms. The histone deacetylase HDA19 interacts with VAL2/HSL1 to repress the expression of embryonic regulators, such as LEC1 and LEC2 via deacetylation of histone H3 (H3Ac) and H4 (H4Ac). The Polycomb group proteins, PRC1 and PRC2, repress the expression of both embryonic and meristematic regulators (WUS, WOX5, and others) through monoubiquitination of H2A at Lys-119 (H2AK119ub) and trimethylation of histone H3 at Lys-27 (H3K27me3), respectively. The VAL1/HSI2 protein physically interacts with At BMI1 and may recruit PRC1 to target loci for their repression. The CHD3/4-like chromatin remodeling protein PKL participates in the deposition of H3K27me3 on the Polycomb targets. In addition, PKL may repress cytokinin response through histone deacetylation. Proteins with confirmed function in callus formation are highlighted with white circles, while those inferred in callus formation based on indirect evidence are unmarked.

and Schmülling, 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 (Krupková and Schmülling, 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 dedifferentiation (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 evolutionally 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 monoubiquitinate 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 meristematic programs 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 generation soon after germination (Chanvivattana et al., 2004; Schubert et al., 2005; Figure 3K). 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 overexpression of embryonic regulators, such as *LEC1*, *LEC2*, *AGL15*, and *BBM*, as well as several meristematic regulators, such as *WUS* and *WUSHEL RELATED HOMEOBOX5* (*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-hypersensitive2*, 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/ABI3-LIKE1 (VAL1; also known as HIGH-LEVEL EXPRRESSION 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 VAL1/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 calli 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.

ACKNOWLEDGMENTS

We thank members of the Sugimoto Lab, especially Christian Breuer, Bart Rymen, Luke Braidwood, and Tetsuya Hisanaga, for helpful discussions and critical reading of the article. This work was supported by Grants-in-Aid for Scientific Research on Innovative Areas (Grant 22119010) and the Programme for Promotion of Basic and Applied Researches for Innovations in Bio-oriented Industry to K.S. A.I. was funded by the RIKEN Special Postdoctoral Researchers Program and by a grant from Japan Society for the Promotion of Science (Grant 24770053).

AUTHOR CONTRIBUTIONS

All authors contributed to writing the article.

Received July 20, 2013; revised July 20, 2013; accepted September 9, 2013; published September 27, 2013.

REFERENCES

Ahuja, M.R. (1965). Genetic control of tumor formation in higher plants. Q. Rev. Biol. **40:** 329–340.

Akiyoshi, D.E., Klee, H., Amasino, R.M., Nester, E.W., and Gordon, M.P. (1984). T-DNA of *Agrobacterium tumefaciens* encodes an enzyme of cytokinin biosynthesis. Proc. Natl. Acad. Sci. USA **81:** 5994–5998.

Akiyoshi, D.E., Morris, R.O., Hinz, R., Mischke, B.S., Kosuge, T., Garfinkel, D.J., Gordon, M.P., and Nester, E.W. (1983). Cytokinin/auxin balance in crown gall tumors is regulated by specific loci in the T-DNA. Proc. Natl. Acad. Sci. USA **80:** 407–411.

Anzola, J.M., Sieberer, T., Ortbauer, M., Butt, H., Korbei, B., Weinhofer, I., Müllner, A.E., and Luschnig, C. (2010). Putative *Arabidopsis* transcriptional adaptor protein (PROPORZ1) is required to modulate histone acetylation in response to auxin. Proc. Natl. Acad. Sci. USA **107:** 10308–10313.

Asahina, M., et al.. (2011). Spatially selective hormonal control of RAP2.6L and ANAC071 transcription factors involved in tissue reunion in *Arabidopsis*. Proc. Natl. Acad. Sci. USA **108:** 16128–16132.

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

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

Barash, I., and Manulis-Sasson, S. (2007). Virulence mechanisms and host specificity of gall-forming *Pantoea agglomerans*. Trends Microbiol. **15:** 538–545.

Berckmans, B., et al. (2011). Auxin-dependent cell cycle reactivation through transcriptional regulation of *Arabidopsis* E2Fa by lateral organ boundary proteins. Plant Cell **23:** 3671–3683.

Bostock, R.M., and Stermer, B.A. (1989). Perspectives on wound healing in resistance to pathogens. Annu. Rev. Phytopathol. **27:** 343–371.

Bourgaud, F., Gravot, A., Milesi, S., and Gontier, E. (2001). Production of plant secondary metabolites: A historical perspective. Plant Sci. **161:** 839–851.

Boutilier, K., Offringa, R., Sharma, V.K., Kieft, H., Ouellet, T., Zhang, L., Hattori, J., Liu, C.-M., van Lammeren, A.A.M., Miki, B.L.A., Custers, J.B.M., and van Lookeren Campagne, M.M. (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., Goodrich, J., Renou, J.-P., Grini, P.E., Colot, V.,

and Schnittger, A. (2011). Polycomb repressive complex 2 controls the embryo-to-seedling phase transition. PLoS Genet. **7:** e1002014.

Bratzel, F., López-Torrejón, G., Koch, M., Del Pozo, J.C., and Calonje, M. (2010). Keeping cell identity in *Arabidopsis* requires PRC1 RING-finger homologs that catalyze H2A monoubiquitination. Curr. Biol. **20:** 1853–1859.

Braun, A.C. (1959). A demonstration of the recovery of the crown-gall tumor cells with the use of complex tumors of single-cell origin. Proc. Natl. Acad. Sci. USA **45:** 932–938.

Burge, C.B., Tuschl, T., and Sharp, P.A. (1999). Splicing of precursors to mRNAs by the spliceosomes. In The RNA World, R.F. Gesteland, T.R. Cech, and J.F. Atkins, eds (Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press), pp. 525–560.

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

Chiappetta, A., Fambrini, M., Petrarulo, M., Rapparini, F., Michelotti, V., Bruno, L., Greco, M., Baraldi, R., Salvini, M., Pugliesi, C., and Bitonti, M.B. (2009). Ectopic expression of *LEAFY COTYLEDON1-LIKE* gene and localized auxin accumulation mark embryogenic competence in epiphyllous plants of *Helianthus annuus* x *H. tuberosus*. Ann. Bot. (Lond.) **103:** 735–747.

Chiappetta, A., Michelotti, V., Fambrini, M., Bruno, L., Salvini, M., Petrarulo, M., Azmi, A., Van Onckelen, H., Pugliesi, C., and Bitonti, M.B. (2006). Zeatin accumulation and misexpression of a class I *knox* gene are intimately linked in the epiphyllous response of the interspecific hybrid EMB-2 (*Helianthus annuus* x *H. tuberosus*). Planta **223:** 917–931.

Chitteti, B.R., and Peng, Z. (2007). Proteome and phosphoproteome dynamic change during cell dedifferentiation in *Arabidopsis*. Proteomics **7:** 1473–1500.

Chitteti, B.R., Tan, F., Mujahid, H., Magee, B.G., Bridges, S.M., and Peng, Z. (2008). Comparative analysis of proteome differential regulation during cell dedifferentiation in *Arabidopsis*. Proteomics **8:** 4303–4316.

Cline, M.N., and Neely, D. (1983). The histology and histochemistry of wound-healing process in geranium cuttings. J. Am. Soc. Hortic. Sci. **108:** 496–502.

Cockcroft, C.E., den Boer, B.G., Healy, J.M., and Murray, J.A. (2000). Cyclin D control of growth rate in plants. Nature **405:** 575–579.

Delessert, C., Wilson, I.W., Van Der Straeten, D., Dennis, E.S., and Dolferus, R. (2004). Spatial and temporal analysis of the local response to wounding in *Arabidopsis* leaves. Plant Mol. Biol. **55:** 165–181.

Deng, W., Luo, K., Li, Z., and Yang, Y. (2009). A novel method for induction of plant regeneration via somatic embryogenesis. Plant Sci. **177:** 43–48.

De Veylder, L., Beeckman, T., Beemster, G.T.S., de Almeida Engler, J., Ormenese, S., Maes, S., Naudts, M., Van Der Schueren, E., Jacqmard, A., Engler, G., and Inzé, D.D. (2002). Control of proliferation, endoreduplication and differentiation by the *Arabidopsis* E2Fa-DPa transcription factor. EMBO J. **21:** 1360–1368.

Dewitte, W., Riou-Khamlichi, C., Scofield, S., Healy, J.M., Jacqmard, A., Kilby, N.J., and Murray, J.A. (2003). Altered cell cycle distribution, hyperplasia, and inhibited differentiation in *Arabidopsis* caused by the D-type cyclin CYCD3. Plant Cell **15:** 79–92.

Dewitte, W., Scofield, S., Alcasabas, A.A., Maughan, S.C., Menges, M., Braun, N., Collins, C., Nieuwland, J., Prinsen, E., Sundaresan, V., and Murray, J.A. (2007). *Arabidopsis* CYCD3 D-type cyclins link cell proliferation and endocycles and are rate-limiting for cytokinin responses. Proc. Natl. Acad. Sci. USA **104:** 14537–14542.

Eckardt, N. (2006). A genomic analysis of tumor development and source-sink relationships in *Agrobacterium*-induced crown gall disease in *Arabidopsis*. Plant Cell **18:** 3350–3352.

El Ouakfaoui, S., Schnell, J., Abdeen, A., Colville, A., Labbé, H., Han, S., Baum, B., Laberge, S., and Miki, B. (2010). Control of somatic embryogenesis and embryo development by AP2 transcription factors. Plant Mol. Biol. **74:** 313–326.

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

Frank, M., Guivarc'h, A., Krupková, E., Lorenz-Meyer, I., Chriqui, D., and Schmülling, T. (2002). Tumorous shoot development (TSD) genes are required for co-ordinated plant shoot development. Plant J. **29:** 73–85.

Frank, M., Rupp, H.-M., Prinsen, E., Motyka, V., Van Onckelen, H., and Schmülling, T. (2000). Hormone autotrophic growth and differentiation identifies mutant lines of Arabidopsis with altered cytokinin and auxin content or signaling. Plant Physiol. **122:** 721–729.

Furuta, K., Kubo, M., Sano, K., Demura, T., Fukuda, H., Liu, Y.G., Shibata, D., and Kakimoto, T. (2011). The CKH2/PKL chromatin remodeling factor negatively regulates cytokinin responses in *Arabidopsis* calli. Plant Cell Physiol. **52:** 618–628.

Gaj, M.D., Zhang, S., Harada, J.J., and Lemaux, P.G. (2005). Leafy cotyledon genes are essential for induction of somatic embryogenesis of *Arabidopsis*. Planta **222:** 977–988.

Gao, P., Xin, Z., and Zheng, Z.-L. (2008). The OSU1/QUA2/TSD2-encoded putative methyltransferase is a critical modulator of carbon and nitrogen nutrient balance response in *Arabidopsis*. PLoS ONE **3:** e1387.

Gaspar-Maia, A., Alajem, A., Meshorer, E., and Ramalho-Santos, M. (2011). Open chromatin in pluripotency and reprogramming. Nat. Rev. Mol. Cell Biol. **12:** 36–47.

Gautheret, R. (1939). Sur la possibilité de réaliser la culture indéfinie des tissues de tubercules de carotte. C. R. Soc. Biol. Paris **208:** 118–120.

George, E.F., and Sherrington, P.D. (1984). Plant Propagation by Tissue Culture. (Eversley, Basingstoke, UK: Exegetics Limited).

Glick, B.R. (1995). The enhancement of plant growth by free-living bacteria. Can. J. Microbiol. **41:** 109–117.

Goren, R., Altman, A., and Giladi, I. (1979). Role of ethylene in abscisic acid-induced callus formation in citrus bud cultures. Plant Physiol. **63:** 280–282.

Grafi, G., Florentin, A., Ransbotyn, V., and Morgenstern, Y. (2011). The stem cell state in plant development and in response to stress. Front Plant Sci. **2:** 53.

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

Hawkins, R.D., et al. (2010). Distinct epigenomic landscapes of pluripotent and lineage-committed human cells. Cell Stem Cell **6:** 479–491.

Heidmann, I., de Lange, B., Lambalk, J., Angenent, G.C., and Boutilier, K. (2011). Efficient sweet pepper transformation mediated by the BABY BOOM transcription factor. Plant Cell Rep. **30:** 1107–1115.

Hemerly, A.S., Ferreira, P., de Almeida Engler, J., Van Montagu, M., Engler, G., and Inzé, D. (1993). cdc2a expression in *Arabidopsis* is linked with competence for cell division. Plant Cell **5:** 1711–1723.

His, I., Driouich, A., Nicol, F., Jauneau, A., and Höfte, H. (2001). Altered pectin composition in primary cell walls of korrigan, a dwarf mutant of *Arabidopsis* deficient in a membrane-bound endo-1,4-beta-glucanase. Planta **212:** 348–358.

Hollender, C., and Liu, Z. (2008). Histone deacetylase genes in *Arabidopsis* development. J. Integr. Plant Biol. **50:** 875–885.

Hu, Y., Bao, F., and Li, J. (2000). Promotive effect of brassinosteroids on cell division involves a distinct CycD3-induction pathway in *Arabidopsis*. Plant J. **24:** 693–701.

Hwang, I., Sheen, J., and Müller, B. (2012). Cytokinin signaling networks. Annu. Rev. Plant Biol. **63:** 353–380.

Ichikawa, T., and Syōno, K. (1988). Tumorization-redifferentiation system of tobacco genetic tumor. Plant Cell Physiol. **29:** 1373–1378.

Ichikawa, T., and Syōno, K. (1991). Tobacco genetic tumors. Plant Cell Physiol. **32:** 1123–1128.

Ikeda, Y., Banno, H., Niu, Q.W., Howell, S.H., and 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 Physiol. **47:** 1443–1456.

Inzé, D., and De Veylder, L. (2006). Cell cycle regulation in plant development. Annu. Rev. Genet. **40:** 77–105.

Ishikawa, M., 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.

Iwai, H., Masaoka, N., Ishii, T., and Satoh, S. (2002). A pectin glucuronyltransferase gene is essential for intercellular attachment in the plant meristem. Proc. Natl. Acad. Sci. USA **99:** 16319–16324.

Iwase, A., Mitsuda, N., Koyama, T., Hiratsu, K., Kojima, M., Arai, T., Inoue, Y., Seki, M., Sakakibara, H., Sugimoto, K., and Ohme-Takagi, M. (2011a). The AP2/ERF transcription factor WIND1 controls cell dedifferentiation in *Arabidopsis*. Curr. Biol. **21:** 508–514.

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

Jammes, F., Lecomte, P., de Almeida-Engler, J., Bitton, F., Martin-Magniette, M.L., Renou, J.P., Abad, P., and Favery, B. (2005). Genome-wide expression profiling of the host response to root-knot nematode infection in *Arabidopsis*. Plant J. **44:** 447–458.

Kehr, A.E. (1951). Genetic tumors in Nicotiana. Am. Nat. **85:** 51–64.

Konishi, M., and Sugiyama, M. (2003). Genetic analysis of adventitious root formation with a novel series of temperature-sensitive mutants of *Arabidopsis thaliana*. Development **130:** 5637–5647.

Kosugi, S., and Ohashi, Y. (2003). Constitutive E2F expression in tobacco plants exhibits altered cell cycle control and morphological change in a cell type-specific manner. Plant Physiol. **132:** 2012–2022.

Koukalova, B., Fojtova, M., Lim, K.Y., Fulnecek, J., Leitch, A.R., and Kovarik, A. (2005). Dedifferentiation of tobacco cells is associated with ribosomal RNA gene hypomethylation, increased transcription, and chromatin alterations. Plant Physiol. **139:** 275–286.

Kőszegi, D., Johnston, A.J., Rutten, T., Czihal, A., Altschmied, L., Kumlehn, J., Wüst, S.E.J., Kirioukhova, O., Gheyselinck, J., Grossniklaus, U., and Bäumlein, H. (2011). Members of the RKD transcription factor family induce an egg cell-like gene expression program. Plant J. **67:** 280–291.

Krupková, E., Immerzeel, P., Pauly, M., and Schmülling, T. (2007). The TUMOROUS SHOOT DEVELOPMENT2 gene of *Arabidopsis* encoding a putative methyltransferase is required for cell adhesion and co-ordinated plant development. Plant J. **50:** 735–750.

Krupková, E., and Schmülling, T. (2009). Developmental consequences of the tumorous shoot development1 mutation, a novel allele of the cellulose-synthesizing KORRIGAN1 gene. Plant Mol. Biol. **71:** 641–655.

Kung, S.D. (1989). Genetic tumors in Nicotiana. Bot. Bull. Acad. Sinica (Taiwan) **30:** 231–240.

Lane, D.R., et al.. (2001). Temperature-sensitive alleles of RSW2 link the KORRIGAN endo-1,4-beta-glucanase to cellulose synthesis and cytokinesis in Arabidopsis. Plant Physiol. **126:** 278–288.

Laux, T., Mayer, K.F.X., Berger, J., and Jürgens, G. (1996). The WUSCHEL gene is required for shoot and floral meristem integrity in *Arabidopsis*. Development **122:** 87–96.

Lee, C. (1955). Anatomical changes in sweet clover shoots infected with Wound-Tumor Virus. Am. J. Bot. **42:** 693–698.

Lee, D.-K., Geisler, M., and Springer, P.S. (2009). LATERAL ORGAN FUSION1 and LATERAL ORGAN FUSION2 function in lateral organ separation and axillary meristem formation in *Arabidopsis*. Development **136:** 2423–2432.

Lotan, T., Ohto, M., Yee, K.M., West, M.A., Lo, R., Kwong, R.W., Yamagishi, K., Fischer, R.L., Goldberg, R.B., and Harada, J.J. (1998). *Arabidopsis* LEAFY COTYLEDON1 is sufficient to induce embryo development in vegetative cells. Cell **93:** 1195–1205.

Malinowski, R., Smith, J.A., Fleming, A.J., Scholes, J.D., and Rolfe, S.A. (2012). Gall formation in clubroot-infected *Arabidopsis* results from an increase in existing meristematic activities of the host but is not essential for the completion of the pathogen life cycle. Plant J. **71:** 226–238.

Manulis, S., Haviv-Chesner, A., Brandl, M.T., Lindow, S.E., and Barash, I. (1998). Differential involvement of indole-3-acetic acid biosynthetic pathways in pathogenicity and epiphytic fitness of *Erwinia herbicola* pv. *gypsophilae*. Mol. Plant Microbe Interact. **11:** 634–642.

Marsch-Martinez, N., Greco, R., Becker, J.D., Dixit, S., Bergervoet, J.H.W., Karaba, A., de Folter, S., and Pereira, A. (2006). BOLITA, an *Arabidopsis* AP2/ERF-like transcription factor that affects cell expansion and proliferation/differentiation pathways. Plant Mol. Biol. **62:** 825–843.

Mayer, K.F., Schoof, H., Haecker, A., Lenhard, M., Jürgens, G., and Laux, T. (1998). Role of WUSCHEL in regulating stem cell fate in the *Arabidopsis* shoot meristem. Cell **95:** 805–815.

Morris, R. (1986). Genes specifying auxin and cytokinin biosynthesis in phytopathogens. Annu. Rev. Plant Physiol. **37:** 509–538.

Mouille, G., Ralet, M.-C., Cavelier, C., Eland, C., Effroy, D., Hématy, K., McCartney, L., Truong, H.N., Gaudon, V., Thibault, J.-F., Marchant, A., and Höfte, H. (2007). Homogalacturonan synthesis in *Arabidopsis thaliana* requires a Golgi-localized protein with a putative methyltransferase domain. Plant J. **50:** 605–614.

Nagata, T., and Takebe, I. (1971). Plating of isolated tobacco mesophyll protoplasts on agar medium. Planta **99:** 12–20.

Neely, D. (1979). Tree wounds and wound closure. J. Arboriculture **5:** 135–140.

Nester, E.W., Gordon, M.P., Amasino, R.M., and Yanofsky, M.F. (1984). Crown gall: A molecular and physiological analysis. Annu. Rev. Plant Physiol. **35:** 387–413.

Nicol, F., His, I., Jauneau, A., Vernhettes, S., Canut, H., and Höfte, H. (1998). A plasma membrane-bound putative endo-1,4-beta-D-glucanase is required for normal wall assembly and cell elongation in *Arabidopsis*. EMBO J. **17:** 5563–5576.

Nobécourt, P. (1939). Sur la pérennité et l'augmentation de volume des cultures de tissues végétaux. Compt. Rendus Soc. Biol. Lyon **130:** 1270–1271.

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

Ogas, J., Kaufmann, S., Henderson, J., and Somerville, C. (1999). PICKLE is a CHD3 chromatin-remodeling factor that regulates the transition from embryonic to vegetative development in *Arabidopsis*. Proc. Natl. Acad. Sci. USA **96:** 13839–13844.

Ohbayashi, I., Konishi, M., Ebine, K., and Sugiyama, M. (2011). Genetic identification of *Arabidopsis* RID2 as an essential factor involved in pre-rRNA processing. Plant J. **67:** 49–60.

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

Okamuro, J.K., Caster, B., Villarroel, R., Van Montagu, M., and Jofuku, K.D. (1997). The AP2 domain of APETALA2 defines a large new family of DNA binding proteins in *Arabidopsis.* Proc. Natl. Acad. Sci. USA **94:** 7076–7081.

Okushima, Y., Fukaki, H., Onoda, M., Theologis, A., and 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., and Sugiyama, M. (1998). Organogenic responses in tissue culture of srd mutants of *Arabidopsis thaliana*. Development **125:** 135–142.

Ralet, M.C., Crépeau, M.J., Lefèbvre, J., Mouille, G., Höfte, H., and Thibault, J.F. (2008). Reduced number of homogalacturonan domains in pectins of an *Arabidopsis* mutant enhances the flexibility of the polymer. Biomacromolecules **9:** 1454–1460.

Ringrose, L., and Paro, R. (2004). Epigenetic regulation of cellular memory by the Polycomb and Trithorax group proteins. Annu. Rev. Genet. **38:** 413–443.

Riou-Khamlichi, C., Huntley, R., Jacqmard, A., and Murray, J.A. (1999). Cytokinin activation of *Arabidopsis* cell division through a D-type cyclin. Science **283:** 1541–1544.

Sacristan, M., and Melchers, G. (1977). Regeneration of plants from "habituated" and "Agrobacterium-transformed" single-cell clones of tobacco. Mol. Gen. Genet. **152:** 111–117.

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

Sanchez-Pulido, L., Devos, D., Sung, Z.R., and Calonje, M. (2008). RAWUL: A new ubiquitin-like domain in PRC1 ring finger proteins that unveils putative plant and worm PRC1 orthologs. BMC Genomics **9:** 308.

Schubert, D., Clarenz, O., and Goodrich, J. (2005). Epigenetic control of plant development by Polycomb-group proteins. Curr. Opin. Plant Biol. **8:** 553–561.

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

Sieberer, T., Hauser, M.-T., Seifert, G.J., and Luschnig, C. (2003). PROPORZ1, a putative *Arabidopsis* transcriptional adaptor protein, mediates auxin and cytokinin signals in the control of cell proliferation. Curr. Biol. **13:** 837–842.

Sitbon, F., Sundberg, B., Olsson, O., and Sandberg, G. (1991). Free and conjugated indoleacetic acid (IAA) contents in transgenic tobacco plants expressing the iaaM and iaaH IAA biosynthesis genes from *Agrobacterium tumefaciens*. Plant Physiol. **95:** 480–485.

Skirycz, A., Radziejwoski, A., Busch, W., Hannah, M.A., Czeszejko, J., Kwaśniewski, M., Zanor, M.I., Lohmann, J.U., De Veylder, L., Witt, I., and Mueller-Roeber, B. (2008). The DOF transcription factor OBP1 is involved in cell cycle regulation in *Arabidopsis thaliana*. Plant J. **56:** 779–792.

Skoog, F., and Miller, C.O. (1957). Chemical regulation of growth and organ formation in plant tissues cultured in vitro. Symp. Soc. Exp. Biol. **11:** 118–130.

Srinivasan, C., Liu, Z., Heidmann, I., Supena, E.D.J., Fukuoka, H., Joosen, R., Lambalk, J., Angenent, G., Scorza, R., Custers, J.B.M., and Boutilier, K. (2007). Heterologous expression of the BABY BOOM AP2/ERF transcription factor enhances the regeneration capacity of tobacco (*Nicotiana tabacum* L.). Planta **225:** 341–351.

Steward, F.C., Mapes, M.O., and Mears, K. (1958). Growth and organized development of cultured cells. II. Organization in cultures grown from freely suspended cells. Am. J. Bot. **45:** 705–708.

Stobbe, H., Schmitt, U., Eckstein, D., and Dujesiefken, D. (2002). Developmental stages and fine structure of surface callus formed after debarking of living lime trees (Tilia sp.). Ann. Bot. (Lond.) **89:** 773–782.

Stone, S.L., Kwong, L.W., Yee, K.M., Pelletier, J., Lepiniec, L., Fischer, R.L., Goldberg, R.B., and Harada, J.J. (2001). LEAFY COTYLEDON2 encodes a B3 domain transcription factor that induces embryo development. Proc. Natl. Acad. Sci. USA **98:** 11806–11811.

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

Tajima, Y., Imamura, A., Kiba, T., Amano, Y., Yamashino, T., and Mizuno, T. (2004). Comparative studies on the type-B response regulators revealing their distinctive properties in the His-to-Asp phosphorelay signal transduction of *Arabidopsis thaliana*. Plant Cell Physiol. **45:** 28–39.

Thakare, D., Tang, W., Hill, K., and Perry, S.E. (2008). The MADS-domain transcriptional regulator AGAMOUS-LIKE15 promotes somatic embryo development in Arabidopsis and soybean. Plant Physiol. **146:** 1663–1672.

Tooker, J.F., Rohr, J.R., Abrahamson, W.G., and De Moraes, C.M. (2008). Gall insects can avoid and alter indirect plant defenses. New Phytol. **178:** 657–671.

Tsukagoshi, H., Morikami, A., and Nakamura, K. (2007). Two B3 domain transcriptional repressors prevent sugar-inducible expression of seed maturation genes in Arabidopsis seedlings. Proc. Natl. Acad. Sci. USA **104:** 2543–2547.

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

Udagawa, M., Aoki, S., and Syono, K. (2004). Expression analysis of the NgORF13 promoter during the development of tobacco genetic tumors. Plant Cell Physiol. **45:** 1023–1031.

Umehara, M., Ikeda, M., and Kamada, H. (2007). Endogenous factors that regulate plant embryogenesis: Recent advances. Jpn. J. Plant Sci. **1:** 1–6.

Valvekens, D., Montagu, M.V., and Van Lijsebettens, M. (1988). *Agrobacterium tumefaciens*-mediated transformation of *Arabidopsis thaliana* root explants by using kanamycin selection. Proc. Natl. Acad. Sci. USA **85:** 5536–5540.

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

Waki, T., Hiki, T., Watanabe, R., Hashimoto, T., and Nakajima, K. (2011). The *Arabidopsis* RWP-RK protein RKD4 triggers gene expression and pattern formation in early embryogenesis. Curr. Biol. **21:** 1277–1281.

White, P.R. (1939). Potentially unlimited growth of excised plant callus in an artificial nutrient. Am. J. Bot. **26:** 59–64.

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

Yordanov, Y.S., Regan, S., and Busov, V. (2010). Members of the LATERAL ORGAN BOUNDARIES DOMAIN transcription factor family are involved in the regulation of secondary growth in *Populus*. Plant Cell **22:** 3662–3677.

Zhang, H., Rider, S.D., Jr., Henderson, J.T., Fountain, M., Chuang, K., Kandachar, V., Simons, A., Edenberg, H.J., Romero-Severson, J., Muir, W.M., and Ogas, J. (2008). The CHD3 remodeler PICKLE promotes trimethylation of histone H3 lysine 27. J. Biol. Chem. **283:** 22637–22648.

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

Zhang, H.M., Yang, J., Xin, X., Chen, J.P., and Adams, M.J. (2007). Molecular characterization of the genome segments S4, S6 and S7 of rice gall dwarf virus. Arch. Virol. **152:** 1593–1602.

Zhao, J., Morozova, N., Williams, L., Libs, L., Avivi, Y., and Grafi, G. (2001). Two phases of chromatin decondensation during dedifferentiation of plant cells: Distinction between competence for

cell fate switch and a commitment for S phase. J. Biol. Chem. **276:** 22772–22778.

Zhou, C., Guo, J., Feng, Z., Cui, X., and Zhu, J. (2012). Molecular characterization of a novel AP2 transcription factor ThWIND1-L from *Thellungiella halophila*. Plant Cell Tissue Organ Cult. **110:** 423–433.

Zhou, Y., et al. (2013). HISTONE DEACETYLASE19 interacts with HSL1 and participates in the repression of seed maturation genes in *Arabidopsis* seedlings. Plant Cell **25:** 134–148.

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., and Chua, N.-H. (2002). The *WUSCHEL* gene promotes vegetative-to-embryonic transition in *Arabidopsis*. Plant J. **30:** 349–359.

Zuo, J., Niu, Q.W., Nishizawa, N., Wu, Y., Kost, B., and Chua, N.H. (2000). KORRIGAN, an *Arabidopsis* endo-1,4-beta-glucanase, localizes to the cell plate by polarized targeting and is essential for cytokinesis. Plant Cell **12:** 1137–1152.