Plant regeneration: cellular origins and molecular mechanisms

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ABSTRACT

Compared with animals, plants generally possess a high degree of developmental plasticity and display various types of tissue or organ regeneration. This regenerative capacity can be enhanced by exogenously supplied plant hormones in vitro, wherein the balance between auxin and cytokinin determines the developmental fate of regenerating organs. Accumulating evidence suggests that some forms of plant regeneration involve reprogramming of differentiated somatic cells, whereas others are induced through the activation of relatively undifferentiated cells in somatic tissues. We summarize the current understanding of how plants control various types of regeneration and discuss how developmental and environmental constraints influence these regulatory mechanisms.

KEY WORDS: Cellular reprogramming, De novo organogenesis, Somatic embryogenesis

Introduction

When living organisms injure or lose part of their bodies, many are able to regenerate new tissues or organs to minimize the impact of local damage. Regeneration is a widely conserved physiological response in both animals and plants (Puliammackal et al., 2014). What is collectively referred to as regeneration can range from the repair of a small amputation to the formation of new organs or individuals, and the mode of regeneration varies markedly among taxa (Birnbaum and Sánchez Alvarado, 2008). Plants possess a high capacity to regenerate, which has long been utilized for clonal propagation and for the generation of new plant bodies.

Regeneration in green algae, liverworts and mosses

Many unicellular green algae have macroscopic bodies, and when these cells are injured they need to repair the damage quickly for survival. Regenerating the whole body directly from damaged cells is unique to these unicellular organisms, as multicellular organisms usually abandon damaged cells and use the remaining intact cells as a source of regeneration. Although most green algae simply seal cut sites by reconnecting the plasma membrane, some algae display astonishing regenerative responses. In the unicellular multinucleated alga *Bryopsis plumosa*, for example, nuclei squeezed out of cut sites aggregate in the seawater and construct the primary membrane *de novo* (Kim et al., 2001) (Fig. 1). These new protoplasts subsequently regenerate the second membrane and eventually rebuild the complete body.

Bryophytes, a group of basal land plants comprising liverworts, hornworts and mosses, have relatively simple body structures and they generally display high regenerative capacities (Necker et al., 1974). Explants of the liverwort *Marchantia polymorpha*, for example, regenerate new apical meristems within 60 h after wounding (Fig. 1) (Nishihama et al., 2015). Regenerating tissues often derive from the ventral midrib near cut ends (Vöchting, 1885), but other cell types also seem to contribute to regeneration (Nishihama et al., 2015). The moss *Physcomitrella patens* regenerates sporelings called protonema from leaf explants (Fig. 1). Protonema are thread-like chains of cells normally produced from spores, but can also be produced within the first 48 h after wounding when leaf cells facing the cut sites change their cell fate into protonema stem cells (Ishikawa et al., 2011). These stem cells subsequently start to elongate and proliferate, leading to the generation of new plant bodies.

Diverse forms of regeneration in seed plants

Compared with basal land plants, seed plants possess more complex body structures and display more diverse modes of regeneration depending on the developmental and/or environmental context (Fig. 2). Seed plants harbor apical meristems at both the top and bottom end of their bodies, known as the shoot and root apical meristem, respectively. The meristems are responsible for repair after injury: when the central zone of the shoot apical meristem is locally ablated, surrounding cells in the peripheral zone reconstruct...
the functional meristem (Reinhardt et al., 2003) (Fig. 2). Similarly, when part of the root meristems is removed, remaining cells in the meristem undergo additional division and rebuild a complete meristem (Sena et al., 2009) (Fig. 2). Plants adopt alternative strategies when entire meristems are excised by macroscopic injuries. In shoots, meristems in axillary buds are kept dormant by the presence of apical meristems. Upon loss of these apical meristems, apical dormancy is broken and axillary buds begin to grow (Shimizu-Sato and Mori, 2001) (Fig. 2). Similarly, when the whole root meristem is removed, new lateral and/or adventitious roots are formed from remaining roots and stems, respectively (Aloni et al., 2006; Bellini et al., 2014) (Fig. 2). In addition to these apical meristems, plant stems also display several types of tissue regeneration, including vascular reformation after debarking (Stobbe et al., 2002), tissue repair after partial incisions (Asahina et al., 2011) (Fig. 2) and vascular reconnection during grafting (Melnyk et al., 2015).

A unique feature of plant regeneration is the formation of new organs from cut sites (Hartmann et al., 2010) (Fig. 2), with the regeneration of roots from shoot cuttings seen in many different plant species. Some limited but phylogenetically diverse plant species, such as those in Crassulaceae (for example Crassula spp., Echeveria spp., Kalanchoe spp. and Sedum spp.), Gesneriaceae (for example Saintpaulia ionantha, Sinningia speciosa and Streptocarpus xhybridus) and others (for example Begonia spp., Peperomia spp. and Sansevieria trifasciata), regenerate both shoots and roots from leaf cuttings (Fig. 2). In some bulbous plants, such as Hippeastrum spp., Hyacinthus spp. and Lilium spp., detached bulb scales regenerate shoots and roots from cut sites (Fig. 2). Some plants, including the perennial herbaceous plants Papaver orientale, Primula sieboldii and Taraxacum officinale, are capable of regenerating shoots from root cuttings (Fig. 2).

The regenerative capacity of plant cells can be enhanced in vitro when explants are cultured on nutrient media supplemented with plant hormones (Skoog and Miller, 1957; Murashige, 1974; George et al., 2008) (Fig. 3). Shoot explants of many ornamental plants are used for clonal propagation because multiple shoots can be formed from a shoot tip or stem node carrying a single bud (Fig. 3). Shoot or

**Fig. 1. Diverse forms of regeneration in green algae, liverworts and mosses.** The unicellular green alga *Bryopsis plumosa* can regenerate complete bodies through the de novo formation of protoplasts. The liverwort *Marchantia polymorpha* can regenerate new meristems (arrowhead) from the cut site. The moss *Physcomitrella patens* can regenerate new protonema cells (arrowheads) from leaf cuttings. Scissors indicate the cut site. Scale bars (from top to bottom): 10 µm, 1 mm, 500 µm. The top photograph is reprinted from Kim et al. (2001) with permission. The middle and bottom photographs were provided by Ryuichi Nishihama and Masaki Ishikawa, respectively.

**Fig. 2. Diverse forms of regeneration in seed plants.** Shoots and roots restore functional apical meristems when part of these meristems is removed (A,F). When they cannot repair existing meristems – for example when the whole meristems are excised – they develop new organs such as axillary shoots and lateral roots (C,E). Plant stems also repair tissues after partial incisions (B). Some plants undergo de novo organogenesis and develop new organs from cut sites (D,G). Photographs (G) show examples of de novo organogenesis, from top to bottom: root regeneration from a shoot cutting of *Dracaena* spp.; shoot regeneration from a leaf cutting of *Haworthia* spp.; shoot and root regeneration from a petiole of African violet *Sinningia ionantha*; shoot and root regeneration from detached bulb scales of *lily Lilium longiflorum*; and shoot regeneration from a root cutting of dandelion *Taraxacum officinale*. Scissors indicate the cut site and arrowheads mark regenerating organs. Scale bars: 5 mm, except 5 cm in the top photograph.
In many cases they also serve as a primary source for shoot regeneration and somatic embryogenesis in vitro (Atta et al., 2009; Che et al., 2007; Tarré et al., 2004) (Fig. 4B). On the other hand, plants can regenerate whole bodies from protoplasts or pollen (Fig. 3), and there are many other examples showing regeneration from differentiated cells. Shoot regeneration, for instance, initiates from mature leaf epidermal cells in Chirita flavimaculata (Nakano et al., 2009) (Fig. 4C) or from stem cortex cells in Chrysanthemum morifolium (Kaul et al., 1990) (Fig. 4D). Likewise, calli that give rise to somatic embryos have a cellular origin clearly distinct from pericycle or vascular cells in Medicago truncatula (Wang et al., 2011). It was reported that epidermal cell fate in developing leaves of Arabidopsis thaliana (Arabidopsis) can also be overwritten by overexpression of the RWP-RK protein RKD4 in order to initiate embryogenesis (Waki et al., 2011) (Fig. 4E). These observations indicate that ‘youth’ is not the prerequisite for plant regeneration and, at least under in vitro culture conditions, fully mature somatic cells can initiate regeneration.

### Wound stress as a trigger for regeneration

Given that most naturally occurring regeneration starts at cut sites (Fig. 2), wound stimuli may in fact provide a primary inductive trigger for this phenomenon (Birnbaum and Sánchez Alvarado, 2008; Ikeuchi et al., 2013; Sugiyama, 2015). In Arabidopsis tissue culture where explants incubated on auxin-rich callus-inducing medium (CIM) were subsequently transferred on to cytokinin-rich shoot-inducing medium (SIM) (Valvekens et al., 1988), intact, uncult plants hardly regenerated shoots at all (Iwase et al., 2015), thus demonstrating a requirement for wound stimuli in initiating regeneration. Wounding induces numerous cellular responses, including the production of plant hormones (Ahkami et al., 2009), loss of cell-to-cell communication and disruption of long-distance signaling (Melnik et al., 2015).

What exactly plants perceive as a wound signal and how they start regeneration are not well understood, but recent studies in Arabidopsis showed that the AP2/ERF-type transcriptional regulator WOUND-INDUCED DEDIFFERENTIATION1 (WIND1) and its homologs WIND2, WIND3 and WIND4 are induced upon wounding and promote callus formation at cut sites (Iwase et al., 2011a,b). Importantly, callus induced by transient overexpression of...
WIND1 regenerates shoots and roots when transferred to non-inducible media (Iwase et al., 2011a), suggesting that WIND1 can reprogram somatic cells to confer pluripotency. Intact Arabidopsis plants ectopically expressing WIND1 regenerate shoots without wounding, and plants expressing the dominant-negative form of WIND1 display reduced efficiency of in vitro shoot regeneration (Iwase et al., 2015). These data support a role for WINDs as key mediators of wound-induced cellular reprogramming (Fig. 5A). Consistent with this, sequential activation of WIND1 and an embryonic regulator, LEAFY COTYLEDON2 (LEC2), induces somatic embryogenesis at both cut and non-cut sites, whereas single activation of LEC2 permits embryogenesis only at cut sites (Iwase et al., 2015). Downstream genes regulated by WINDs are currently unknown, although WINDs have been implicated in the control of cytokinin signaling based on the observation that the repression of WIND activity abolishes the wound-induced cytokinin response (Iwase et al., 2011a,b). Further investigation of how wounding activates WIND gene expression and how, in turn, WINDs promote cellular reprogramming will be crucial to advance our molecular understanding of wound-induced regeneration. Precisely which cell types contribute to naturally occurring regeneration is also not fully established, and thus future studies should clarify the origin of cells in natural regeneration and determine whether it involves fate conversion of fully differentiated somatic cells and/or the activation of existing competent cells.

**Molecular basis of de novo shoot organogenesis**

As in many other plant species, Arabidopsis explants do not readily regenerate shoots, but incubation in CIM and SIM strongly enhances shoot regeneration from pericycle cells (Valvekens et al., 1988; Che et al., 2007; Atta et al., 2009). Recent histological and transcriptome analyses have revealed that CIM-induced callus resembles lateral root meristem, which is competent to regenerate shoots upon transfer to SIM (Che et al., 2007; Atta et al., 2009; Sugimoto et al., 2010; Duclercq et al., 2011). Induction of the AP2/ERF transcription factors PLETHORA3 (PLT3), PLT5 and PLT7 is among the earliest transcriptional responses induced by CIM, which in turn leads to the activation of the key root meristem regulators PLT1 and PLT2 to establish a pluripotent root meristem-like callus (Kareem et al., 2015). Expression of PLT3, PLT5 and PLT7 also induces the NAC family transcription factors CUPSHAPED COTYLEDON1 (CUC1) and CUC2, which are involved in shoot meristem initiation during zygotic embryogenesis (Kareem et al., 2015; Aida et al., 1997, 1999). The plt3 plt5 plt7 triple mutants are defective in shoot regeneration, but dual overexpression of PLT1 and CUC2, but not their single expression, partially complements this phenotype, suggesting that PLT3, PLT5 and PLT7 promote both PLT1-mediated acquisition of pluripotency and CUC2-mediated initiation of shoot fate (Kareem et al., 2015) (Fig. 5A). Both CUC1 and CUC2 are uniformly expressed in CIM-induced callus (Gordon et al., 2007) and their presence is associated with cellular pluripotency in various experimental conditions (Cary et al., 2002; Daimon et al., 2003; Gordon et al., 2007; Motte et al., 2011).

Upon transfer to SIM, partitioning of the auxin and cytokinin responses in the pluripotent cell mass is thought to refine the shoot meristem fate. CUC2 expression is restricted to the low cytokinin response domains, whereas the shoot meristem regulator WUSCHEL (WUS) is induced in the high cytokinin response domains (Gordon et al., 2007; Chatfield et al., 2013; Che et al., 2006) (Fig. 5A). The CUC2-expressing cells continue to proliferate to form dome-like structures called promeristems, in which localized upregulation of other regulators such as PIN-FORMED1 (PIN1) and SHOOT MERISTEMLESS (STM) further defines the radial patterning of newly developing meristems and primordia initiation (Gordon et al., 2007). Similar to WUS, other AP2/ERF transcription factors, such as ENHANCER OF SHOOT REGENERATION/DORNROŠCHEN (ESR1/DRN) and ESR2/DRN-LIKE (DRNL), which control embryonic patterning and
Molecular basis of de novo root organogenesis

Culturing Arabidopsis explants on CIM and root-inducing medium (RIM) strongly enhances root regeneration from pericycle cells (Ozawa et al., 1998; Che et al., 2002). This is probably because CIM promotes the production of a root meristem-like pluripotent cell mass, which then becomes further specified by RIM to develop root meristems. Consistent with this, root explants, which already possess lateral root meristem primordia along their body axis, regrow roots from both cut and non-cut sites without pretreatment on CIM, whereas hypocotyl explants regenerate roots only from cut sites under these conditions (Ozawa et al., 1998). Pretreatment of hypocotyl explants on CIM allows root regeneration from non-cut sites (Ozawa et al., 1998), confirming the physiological role of CIM in endowing regenerative competence.

Some plant species naturally regenerate roots from cuttings and several plant hormones, including auxin and cytokinin, are known to control this process (da Costa et al., 2013; Bellini et al., 2014). A recent study by Liu et al. (2014) uncovered a novel molecular link connecting auxin accumulation at cut sites to the formation of new root meristems during regeneration. When Arabidopsis leaves are detached, accumulation of auxin at cut sites induces the expression of two homeobox transcription factors, namely WUSCHEL RELATED HOMEOBOX11 (WOX11) and WOX12, in the procambium and surrounding parenchyma cells. Expression of these genes promotes the fate conversion from leaf procambium/parenchyma cells to root founder cells (Fig. 5B). Both WOX11 and WOX12 subsequently participate in the de novo establishment of root meristems, which further involves the expression of LATERAL ORGAN BOUNDARIES DOMAIN16 (LBD16), LBD29 and WOX5 (Liu et al., 2014) (Fig. 5B). The LBDs and WOX5 are also involved in lateral root development, in which locally accumulated auxin promotes the formation of new root meristems from pericycle cells (Goh et al., 2012; Ditengou et al., 2008). These two pathways thus share some key regulators to facilitate the auxin-mediated establishment of root meristems. Of note, the induction of WOX11 in root regeneration requires auxin response elements (AuxREs) in its promoter, suggesting that some members of the AUXIN RESPONSE FACTOR (ARF) family directly activate WOX11 expression in leaves (Liu et al., 2014).

Molecular basis of somatic embryogenesis

Some somatic cells in plants can restart embryogenesis in vitro when they are exposed to a wide range of severe abiotic stressors (Fehér, 2014). Somatic embryogenesis can be induced by salt, hypochlorite, osmotic pressure, heavy metal ions or high temperature in Daucus carota (Kiyosue et al., 1989, 1990;...
Kamada et al., 1989, 1993, 1994), and similar stress-induced embryogenesis has also been reported in Arabidopsis [Ikeda-Iwai et al., 2003]. Many plant species also undergo somatic embryogenesis when they are cultured on auxin-containing medium and then transferred to auxin-free medium (Wernicke and Brettell, 1980; Lu et al., 1983; Ikeda-Iwai et al., 2002). Among several synthetic auxin-like substances, 2,4-dichlorophenoxyacetic acid (2,4-D) is the most effective inducer of somatic embryos in many plants, possibly because it triggers both auxin and stress responses simultaneously (Gliviwicka et al., 2013). During indirect somatic embryogenesis, by which most somatic embryos are formed, high levels of auxin in the culture medium first promote cell proliferation and embryonic callus formation (Ikeda-Iwai et al., 2002). A key physiological event after the transfer to auxin-free medium is the de novo establishment of auxin gradients in the embryonic callus. This initiates a developmental program similar to zygotic embryogenesis, and is also guided by polarized auxin distribution (Liu et al., 1993; Su et al., 2009). These auxin gradients subsequently lead to the localization of WUS expression to low auxin response domains, marking the position of future shoot meristem formation (Su et al., 2009) (Fig. 5C).

Several key regulators of zygotic embryogenesis and seed development, including the CCAAT box-binding transcription factor LEAFY COTYLEDON1 (LEC1), the B3 domain transcription factors LEC2 and FUSCA3 (FUS3), and the MADS box transcription factor AGAMOUS-LIKE15 (AGL15), are subsequently induced during somatic embryogenesis and control several downstream physiological responses to promote embryonic development (Braybrook and Harada, 2008). A key consequence of this transcriptional reprogramming is the further refinement of auxin production and signaling. LEC1 induces the YUC4A10 (YUC10) gene, which encodes an auxin biosynthesis enzyme, and LEC2 activates the YUC2 and YUC4 genes (Junker et al., 2012; Stone et al., 2008). LEC2 and AGL15 promote the expression of INDOLE ACETIC ACID INDUCIBLE30 (IAA30), a negative regulator of auxin signaling, to modulate the auxin-mediated signaling (Braybrook et al., 2006; Zheng et al., 2009). Previous studies also suggest that a low level of gibberellin (GA) relative to abscisic acid (Braybrook et al., 2006; Zheng et al., 2009). In addition, FUS3 downregulates GA biosynthesis by repressing GA3ox1 and GA3ox2, and induces YUC10 transcriptional changes will be an important task for future studies.

Epigenetic control of regeneration

The regenerative capacity of plant cells is required only when they experience damage. Recent studies have shown that several epigenetic mechanisms actively suppress regenerative potential during normal development (Ikeuchi et al., 2015a). POLYCOMB REPRESSIVE COMPLEX2 (PRC2) is a chromatin modifier that maintains transcriptional repression through the deposition of histone H3 lysine 27 trimethylation (H3K27me3) (Holec and Berger, 2012). A recent study showed that PRC2 mutants initially develop wild-type-like roots with fully differentiated, endoreplicated root hair cells, but that they subsequently reprogram and develop callus and embryo-like structures (Ikeuchi et al., 2015b). The reprogramming regulator WND3 and the embryonic regulator LEC2 are among the key targets repressed by PRC2 in this context and elevated expression of these genes contributes to cellular reprogramming in PRC2 mutants. This study thus enforces the idea that highly differentiated cells still retain the capacity to undergo embryogenesis, and that this potential must be tightly regulated – in this case, epigenetically repressed by PRC2 to maintain the differentiated status. Intriguingly, many other key regulators of regeneration, such as WOX11, WOX5, WUS and STM, are also under PRC2-mediated repression (Liu et al., 2014, 2011; He et al., 2012; Lafos et al., 2011). An important question is whether the cells carrying these repressive marks on regeneration regulators initiate regeneration in the wild-type context and, if so, how these repressions are relieved to allow regeneration to proceed in nature or in vitro conditions. PRC2 is also required for root regeneration from leaves (Liu et al., 2014), suggesting that repression of original cell fate might be another important aspect of regeneration.

Histone deacetylation, which is also implicated in transcriptional repression, might serve as another safeguard to prevent the untimely onset of somatic embryogenesis. Wild-type Arabidopsis plants treated with an inhibitor of histone deacetylases, trichostatin A (TSA), produce embryo-like structures from true leaves (Tanaka et al., 2008). Similarly, loss-of-function mutants of two histone deacetylases, HDA19 and HDA6, generate embryo-like structures in shoots (Tanaka et al., 2008). These phenotypes are associated with the ectopic expression of several key embryonic regulators, such as LEC1 and LEC2, and can be suppressed by introducing the lec1 mutation (Tanaka et al., 2008). Interestingly, TSA in combination with heat treatment greatly enhances the efficiency of somatic embryogenesis from Brassica napus microspores (Li et al., 2014). It is plausible, then, that heat stress and histone deacetylation converge on the upregulation of embryonic regulators to initiate the embryonic program.

Genetic studies in Arabidopsis also suggest the involvement of other epigenetic mechanisms in the control of organ regeneration. Mutations in DNA METHYLTRANSFERASE1 (MET1) enhance shoot regeneration on SIM and this phenotype is accompanied by the elevated expression of several MET1 targets, including WUS (Li et al., 2011). As with many other regeneration regulators, the WUS locus is marked by several other epigenetic signatures and these marks are modified when WUS expression is upregulated during shoot regeneration (Li et al., 2011). Uncovering the causal relationships between these epigenetic modifications and transcriptional changes will be an important task for future studies.

Natural variations that impact plant regeneration

Small genetic variations within the same species can cause dramatic differences in the regenerative response. Genetic variability has been utilized to identify novel factors that modulate the efficiency of regeneration in many plant species (Armstrong et al., 1992; Taguchi-Shiobara et al., 1997; Ben Amer et al., 1997; Flores Berrios et al., 2000; Mano and Komatsuda, 2002; Trujillo-Moya et al., 2011). A leucine-rich repeat receptor-like kinase, RECEPTOR-LIKE PROTEIN KINASE1 (RPK1), for instance, was identified as a major quantitative trait locus (QTL) that affects shoot regeneration in Arabidopsis accessions (Motte et al., 2014). RPK1 is implicated in ABA signaling, and although this hormone has not been studied extensively in the context of regeneration it has been reported to influence shoot regeneration in several plant species (Ghasemi Bezdi et al., 2007; Hoang and Raaldugina, 2012; Huang et al., 2012). The single-nucleotide polymorphism responsible for the genetic variation lies within a putative ligand-binding domain, and thus the identification of its ligands should help to reveal its molecular functions.

In Oryza sativa, some varieties or even cultivars within the same variety exhibit markedly different shoot regeneration capabilities.
Map-based cloning using the low regeneration cultivar Koshihikari (*Japonica*) and high regeneration cultivar Kasalath (*Indica*) identified a ferredoxin-nitrite reductase as a major QTL causing variations in shoot regeneration. Further studies showed that the reductase activity positively correlated with regeneration capacity in several other *Japonica* cultivars as well (Nishimura et al., 2005). Furthermore, introduction of the ferredoxin-nitrite reductase gene from Kasalath improved shoot regeneration in Koshihikari (Nishimura et al., 2005). Ferredoxin-nitrite reductase is involved in the nitrogen assimilation pathway, and thus it might be that low reductase activity in Koshihikari results in an accumulation of nitrite, which might hinder shoot regeneration.

The *Regeneration1* (*Rg1*) locus in tomato was originally identified as a natural variation responsible for highly efficient shoot regeneration in the wild relative *Solanum peruvianum* (Koornneef et al., 1993). It was later shown that *Rg1* increases the competency for both root and shoot regeneration, and that this response does not involve alterations in auxin sensitivity or competency for both root and shoot regeneration, and that this is caused by the loss of auxin responsiveness in reproductive shoots (Rasmussen et al., 2015). By contrast, application of auxin improves the root regeneration responsiveness in reproductive shoots (Rasmussen et al., 2015). By contrast, application of auxin improves the root regeneration responsiveness in reproductive shoots (Rasmussen et al., 2015). By contrast, application of auxin improves the root regeneration responsiveness in reproductive shoots (Rasmussen et al., 2015). By contrast, application of auxin improves the root regeneration responsiveness in reproductive shoots (Rasmussen et al., 2015). By contrast, application of auxin improves the root regeneration responsiveness in reproductive shoots (Rasmussen et al., 2015).

**Developmental constraints that impact plant regeneration**

The regenerative capacity of explants varies markedly with the condition of parental plants, generally declining as plants get older. Compromised root regeneration in aged trees is a serious problem in horticulture, limiting the clonal propagation of elite cultivars. Histological studies using woody species *Castanea sativa* and *Quercus* sp. showed that the exogenous application of auxin reactives cell proliferation but not the formation of new root meristems in mature explants (Ballester et al., 1999; Vidal et al., 2003). A recent study using *Pisum sativum* suggested that the vegetative-to-reproductive transition is linked to the reduced root regenerative capacity and that this is caused by the loss of auxin responsiveness in reproductive shoots (Rasmussen et al., 2015). By contrast, application of auxin improves the root regeneration efficiency of *Arabidopsis* leaves from aged plants (Chen et al., 2014), suggesting that there are multiple physiological constraints imposed by aging.

Explants from juvenile plants regenerate shoots more effectively than those from mature plants (Dong and Jia, 1991; Baker and Bhatia, 1993; Becerra et al., 2004; Zhang et al., 2015). The decline in shoot regeneration capacity with aging is at least partly due to a reduced responsiveness to plant hormones. The microRNA miR156 has been shown to regulate the juvenile-to-adult phase transition in plants (Wu et al., 2009), and a recent study suggests that a decline of miR156 expression in old plants is responsible for reduced shoot regeneration (Zhang et al., 2015). This reduction in miR156 increases the level of its target *SQUAMOSA PROMOTER BINDING-LIKE9* (*SPL9*). SPL9, in turn, inhibits the transcriptional activity of B-type *ARABIDOPSIS RESPONSE REGULATOR* proteins (ARRs), leading to the reduced responsiveness to cytokinin and hence compromised shoot regeneration (Zhang et al., 2015).

**Environmental constraints that impact plant regeneration**

The regenerative capacity of plant explants is also influenced by various environmental conditions, such as nutrient composition, gelling agents, pH, light and temperature (George et al., 2008). A well-documented environmental condition that influences plant regeneration is the exposure to light, but its impact on regeneration appears to be highly context dependent. Light is required for shoot regeneration in some plant species (Reuveni and Evenor, 2007) and can also trigger organ regeneration (Saitou et al., 1992; Sorin et al., 2006; Gutierrez et al., 2009). On the other hand, exposure to light can have an inhibitory effect on root or shoot regeneration in some contexts (Bellini et al., 2014; Nameth et al., 2013). A series of tissue culture experiments using *Arabidopsis* cotyledons showed that light exposure during the first hours after tissue excision is mostly deleterious to shoot regeneration, and keeping explants in darkness for as little as 2-6 h is sufficient to improve regeneration (Nameth et al., 2013).

Light exposure invokes several parallel signaling pathways, some of which cause oxidative damage due to the production of reactive oxygen species. At least two photoreceptors are implicated in the light response of shoot regeneration: the blue/UV-A light receptor CRYPTOCHROME1 (*CRY1*), which mediates the strong inhibition of shoot regeneration; and the far-red light receptor PHYTOCHROME A (*PHYA*), which protects explants against initial light inhibition (Nameth et al., 2013). A key regulator acting downstream of light signaling is the transcription factor ELONGATED HYPOCHOTYL5 (*HY5*), which appears to protect explants against light exposure by inducing anthocyanin accumulation. Several accessions in *Arabidopsis* display different responses to light in shoot regeneration, and an interesting topic for future studies will be the cause of such genetic variations.

**Conclusions and future perspectives**

During regeneration, select intrinsic developmental programs are ectopically activated in response to external stimuli. These responses require context-dependent integration of developmental and environmental signals, leading to diverse strategies and efficiencies of regeneration. Given that regeneration originates from a relatively small population of cells in somatic tissues, it is important to identify these cell populations and to study how external stress can cause them to undergo changes in cell fate. During normal development, many central regulators of regeneration are epigenetically silenced to prevent inappropriate cellular reprogramming. A central challenge, therefore, is to understand how these repressions are overcome by external stimuli. Molecular genetic studies in *Arabidopsis* have provided substantial insight into how plants regenerate from relatively undifferentiated cells, but other plants that regenerate from differentiated cells may utilize distinct mechanisms. With rapid advances in next-generation sequencing and genome editing technologies, we should be able to investigate the molecular mechanisms of these currently underexplored forms of regeneration and carry out functional studies in non-model plants. The QTL analysis on accessions with differing regeneration efficiencies has proved an excellent complementary approach and the further identification of new QTLs should help us to uncover novel mechanisms of plant regeneration.

An important goal of plant regeneration research is to use our knowledge of basic biology to design new molecular tools to analyze and improve regeneration efficiencies in crops. Indeed, the ectopic expression of key regeneration regulators, such as WUS and WIND1, has already been shown to promote organ regeneration and/or somatic embryogenesis in various crops (Srinivasan et al., 2007; Arroyo-Herrera et al., 2008; Heidmann et al., 2011; Iwase et al., 2013, 2015; Florez et al., 2015). Expression profiles of key...
regeneration regulators have also been used in crops to identify cultivars with high regeneration capacities (Malik et al., 2008). Further mechanistic understanding of plant regeneration should help us to advance the classic but not fully exploited field of tissue culture, with numerous downstream implications for both basic and applied biology.

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Competing interests

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