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PRIMER

Model systems for regeneration: *Arabidopsis*

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ABSTRACT

Plants encompass unparalleled multi-scale regenerative potential. Despite lacking specialized cells that are recruited to injured sites, and despite their cells being encased in rigid cell walls, plants exhibit a variety of regenerative responses ranging from the regeneration of specific cell types, tissues and organs, to the rebuilding of an entire organism. Over the years, extensive studies on embryo, shoot and root development in the model plant species *Arabidopsis thaliana* have provided insights into the mechanisms underlying plant regeneration. These studies highlight how *Arabidopsis*, with its wide array of refined molecular, genetic and cell biological tools, provides a perfect model to interrogate the cellular and molecular mechanisms of reprogramming during regeneration.

KEY WORDS: *Arabidopsis*, Auxin, Regeneration, Root, Shoot

Introduction

All living organisms share an inherent ability irrespective of their evolutionary origin, i.e. the ability to grow. In animals, growth is broadly characterised as the enlargement of a body plan that is established during embryogenesis. However, plants make a very limited set of structures during embryogenesis, and most of the plant organs that we see (e.g. the shoot and root) are made and grown post-embryonically. This growth is driven by groups of highly dividing cells, termed meristematic cells, located at the oppositely placed poles of the plant body axis: the shoot apical meristem (SAM; see Glossary, Box 1) and the root apical meristem (RAM; see Glossary, Box 1). The two meristems harbour a region called the stem cell niche (SCN) in which stem cells are confined. These stem cells act as self-perpetuating reservoirs of cells for making various organs during post-embryonic development. Plant growth and survival are compromised when these post-embryonic structures encounter frequent injuries, be it biotic or abiotic. To surpass the damaging effects of these injuries, plants elicit prompt regenerative responses.

Unlike most animals, in which regenerative responses are restricted to specific cell lineages, plants exhibit responses that are quite ubiquitous. As such, plants are distinct by virtue of their ability to regenerate specific cell types, tissues and organs, or even an entire organism. In addition, plants have the remarkable ability to rejoin two different body parts from two individual plants of different origin via grafting to form a 'chimera' (see Glossary, Box 1) that displays the phenotype of both plants. Most importantly, plants display unparalleled plasticity despite the lack of cell migration, which is often key to regeneration in animals. Thus, the extreme fantasies of regeneration paraded by many mythical and comic characters are brought to life by one single life form: the plant.

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Regeneration has been studied in a variety of plant species, revealing varying degrees of regenerative capacities and modes of regeneration (see Box 2). Here, we provide an overview of how an array of regenerative responses are being studied using the model plant species *Arabidopsis thaliana*, and how rapidly growing genetic, cellular and genomic tools have begun to unravel the mechanisms underlying these regenerative responses. We also introduce how a combination of experimental and computational approaches can allow us to gain deeper insights into broader questions in the area of plant regeneration.

Arabidopsis as a model plant

Arabidopsis thaliana (Fig. 1), by virtue of its small genome size, ease of cultivation, short life-span and prolific seed production, has long served as a model in plant molecular and genetic studies. In recent years, the use of *Arabidopsis* has been substantiated by extensive physical and genetic maps of its chromosomes, high-resolution expression maps in space and time, and spatio-temporal studies of various molecular interactions (e.g. protein-protein interactions and protein-DNA interactions) at cellular resolution in its shoot and root (Brady et al., 2007; Busch et al., 2012; Cui et al., 2007; Kulkarni et al., 2018; Weigel and Mott, 2009). In addition to a vast repository of mutant lines, full length cDNAs and miRNA collections, inducible knockdowns and knockouts of desired genes using RNAi and CRISPR-Cas9 have been created and made available (Mao et al., 2019; Schwab et al., 2006). A recently developed technology, termed Inducible Genome Editing (IGE), which knocks out target genes in specific cell types at any developmental age, has further advanced the strengths of *Arabidopsis* as a model plant (Wang et al., 2020a).

The genome-wide binding of a variety of key regulators and their direct downstream targets has also been made available in *Arabidopsis* using DAP-seq, a high-throughput transcription factor (TF) binding site discovery method (O'Malley et al., 2016). In addition, highly sensitive sensors that can measure fluctuations in hormone levels in *Arabidopsis* have been developed (Brunoud et al., 2012; Kang et al., 2012; Liao et al.,

Model systems for regeneration

This article is part of a series entitled 'Model systems for regeneration'. This series of articles aims to highlight key model systems and species that are currently being used to study tissue and organ regeneration. Each article provides background information about the phylogenetic position of the species, its life-cycle and habitat, the different organs and tissues that regenerate, and the experimental tools and techniques that are available for studying these organisms in a regenerative context. Importantly, these articles also give examples of how the study of these models has increased our understanding of regenerative mechanisms more broadly, and how some of the open questions in the field of regeneration may be answered using these organisms. To see the full collection as it grows, please visit: https://dev.biologists.org/collection/regeneration_models.

Development (2021) 148, dev195347. doi:10.1242/dev.195347

Box 1. Glossary of terms

Callus. A pluripotent mass of cells, derived from adult stem cells, which can give rise to an entire plant *de novo* upon application of the appropriate growth hormones.
Chimera. An organism in which cells of two different genotypes exist and grow side by side. Such a plant, which is generated by grafting together two plants of different origins, displays properties of both its donors.
Dedifferentiation. The process by which a differentiated cell loses its identity and exists in an unspecified state.
Pluripotent. Harbouring the ability to give rise to any cell types of the shoot and/or root system.
Quiescent centre (QC). A small group of inert cells in the root apical meristem that occasionally divides to maintain the stem cell pool.
Root apical meristem (RAM). Part of the root tip (primary or lateral root) comprising highly dividing cells called meristematic cells and a confined stem cell niche (SCN).
Shoot apical meristem (SAM). Part of the shoot tip comprising highly dividing cells called meristematic cells and a confined SCN.
Somatic embryogenesis. The development of adventitious or ectopic embryos that arise from somatic cells and have the potential to develop into new plants.
Suspensor. A zygotic structure that provides physical support, nutrition and growth regulators to the embryo, and pushes the embryo into the nutrient-rich endosperm.
Trans-differentiation. The direct fate conversion of cells of one lineage to another without passing through an intermediary dedifferentiated stage.

2015). The readily available molecular, genetic and cell biological tools, together with computational models and the wealth of knowledge of embryonic shoot and root development, has made *Arabidopsis* the perfect choice for studying the mechanisms of cellular reprogramming during normal development as well as during regeneration (Cruz-Ramírez et al., 2012; Grieneisen et al., 2007; Mündermann et al., 2005; Sampathkumar et al., 2014; Smith et al., 2006).

Types of regenerative modes in *Arabidopsis*

Arabidopsis shows a repertoire of regenerative responses that can be as elaborate as regenerating an entire plant from a small tissue or regenerating a lost organ, or as simple as inducing a wound healing response following mechanical injury. These modes of regeneration fall into two broad categories, as highlighted below.

Tissue culture-induced regeneration

Plants can regenerate themselves from various explants of different developmental origins. An explant is a piece of tissue or organ that can be cultured *in vitro* to regenerate organs or an organism *de novo*. This property of explants to produce entire organisms can be exploited for large-scale seedling production. Here, an entire plant is regenerated through the sequential regeneration of shoots and roots. Formation of such organs from explants *in vitro* is called *de novo* organogenesis, and can further be classified into *de novo* shoot regeneration (i.e. shoot formation) and *de novo* root regeneration (i.e. root formation). *De novo* organogenesis can occur via two different pathways, namely direct and indirect regeneration. During indirect regeneration, the explants produce a shoot/root via an intermediary stage called a callus (see Glossary, Box 1; Fig. 2). In direct regeneration, by contrast, the explants can bypass this callus stage to produce a shoot/root. For example, shoot regeneration can occur from lateral root primordia (LRP) (Fig. 3A,B).

In addition to shoots and roots, explants can regenerate embryos and thereby the entire plant system. Here, somatic cells from explants can be induced and cultured *in vitro* to generate somatic embryos via the process of somatic embryogenesis (see Glossary, Box 1). Similar to *de novo* organogenesis, somatic embryogenesis can also occur indirectly via a callus or directly from the explant (Fig. 3C) (Mordhorst et al., 1998; Pillon et al., 1996).

Mechanical injury-induced regeneration

Plants are constantly subjected to mechanical injuries by biotic factors such as herbivores, insects and nematodes, as well as abiotic factors such as strong winds and heavy rains. Mechanical injury-induced regeneration can be exhibited by an organ in two scenarios: (1) when the damaged organ is still attached to the parent plant; (2) when the organ is detached from the parent plant. The most common scenario involves the functional restoration of a damaged organ that is attached to the growing plant. Both aerial and underground organs are capable of combating these injuries. Injuries to aerial organs include vascular disruption inflicted by sap-feeding insects, breakage of stems by strong winds, or even surface abrasion due to friction. Friction-induced surface abrasion usually does not pose a major threat and is healed by local cell proliferation. However, injury to the plant vasculature impedes the transport of water and nutrients along the plant body axis, and prompt re-establishment of vascular continuity is therefore imperative. Injuries to the root are often caused by soil-dwelling nematodes. The injuries range from damage of individual cells by the proboscis of insects to even the loss of an entire root tip. As roots mediate the

Box 2. Regeneration in other plant species

The phenomenon of regeneration, often observed in multicellular organisms, extends across the plant kingdom and to unicellular plants. For example, *Bryopis plumosa* – a unicellular marine green alga – extrudes its protoplast when damaged underwater; the naked protoplast later develops a cell wall and becomes a whole organism (Kim et al., 2001). In basal plants such as *Physcomitrella patens* (a moss), the excised distal half of a gametophore leaf develops a protonema from its cut end (Ishikawa et al., 2011). The protonema is a juvenile filamentous structure that later develops adult gametophores. Similarly, several higher plants exhibit shoot/root regeneration from the cut ends of detached leaves (reviewed by Ikeuchi et al., 2016). The fate respecification in these cases is analogous to the cell fate changes occurring at the cut ends of a detached *Arabidopsis* leaf.

Regeneration studies in angiosperms have gained popularity with the advent of tissue culture. Nearly all *Arabidopsis* explants can be induced to form a pluripotent callus and, subsequently, to elicit shoot regeneration. However, in monocots like rice (*Oryza sativa*), a callus is initiated only from certain explants, such as the base of young leaves, root tips and lateral root-forming regions (Hu et al., 2017). Such differences in cellular plasticity are attributed to species-specific intrinsic perturbations in molecular mechanisms. Nevertheless, the activation of root stem cell regulators that confer callus pluripotency is common to *Arabidopsis* and the tree species, Poplar, suggesting that the mechanism of pluripotency acquisition is conserved (Liu et al., 2018). In addition, genome-wide gene expression profiling has revealed that the tissue culture-derived callus of *Agave salmiana* expresses orthologues of various *Arabidopsis* callus-inducing genes (Cervantes-Pérez et al., 2018). Regeneration of xylem around severed vasculature, as seen in *Arabidopsis*, has also been observed in Coleus internodes and in pea seedlings, and can be explained by the 'auxin canalization hypothesis', whereby auxin flux feeds back on the polar localization of its own transporter (Jacobs, 1952; Sachs, 1969, 1981, 1991). A similar mechanism operates to reinstate vascular continuity in injured *Arabidopsis* stems and leaves. Together, these findings highlight that some aspects of regeneration are conserved in plants, although many species-specific variations are likely to exist.

Development (2021) 148, dev195347. doi:10.1242/dev.195347

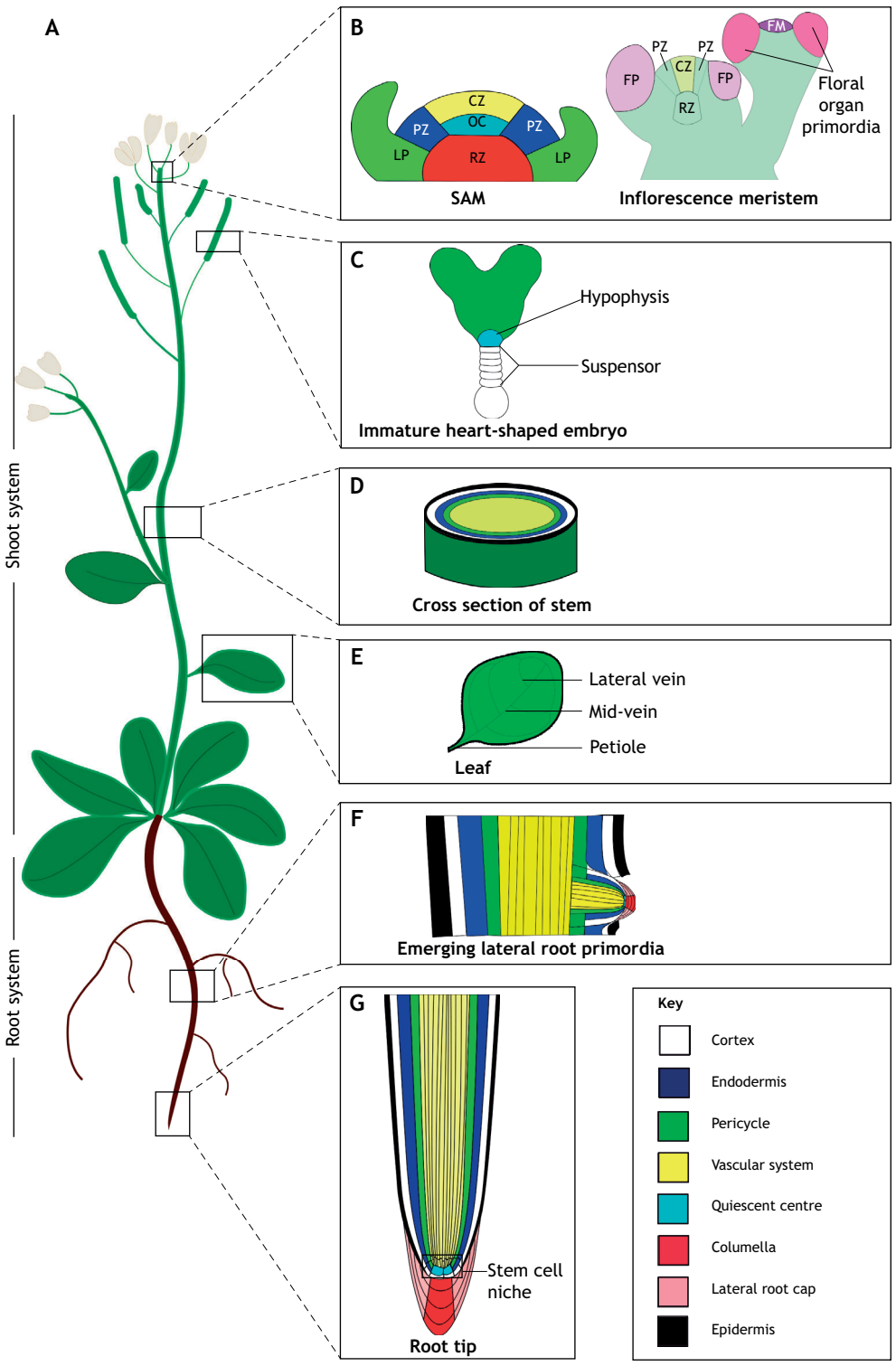


Fig. 1. *Arabidopsis* plant architecture. (A) Representative image of a whole plant. (B) Upon transition from the vegetative phase to the reproductive phase, the shoot apical meristem (SAM), which produces a leaf at its flank, becomes an inflorescence meristem and produces a flower at its flank. The organizing centre (OC) and the central zone (CZ) within the SAM constitute the stem cell niche (SCN). (C) Inset depicts an immature heart-shaped embryo within the silique (fruit). (D) Inset displays a cross-section of the inflorescence stem showing different tissues. (E) Inset shows the leaf with mid vein, lateral vein and petiole. (F) Inset depicts lateral root emergence. (G) Inset depicts the root tip showing different tissues. The quiescent centre and its surrounding cells form the SCN within the root apical meristem. FM, floral meristem; FP, floral primordia; LP, leaf primordia; PZ, peripheral zone; RZ, rib zone.

absorption of water and nutrients, plants initiate immediate defence and regenerative responses to repair this damage.

In contrast to an attached organ, a detached organ exhibits entirely different regeneration responses. Instead of repair and functional restoration of the existing tissue, a detached organ has the ability to regenerate a new organ with an entirely different identity from its cut end. For example, *de novo* root regeneration can occur from the cut ends of detached *Arabidopsis* leaves. Interestingly, a detached organ from one plant can be attached to another plant via the process of grafting to generate a single fully functional plant with characteristics from both the combining plants.

Tissue culture-induced regeneration

De novo shoot regeneration via callus formation

An entire plant can be regenerated from explants of different developmental origin (Glazebrook and Weigel, 2002). These explants, when incubated with synthetic auxin, produce a pluripotent (see Glossary, Box 1) callus from xylem pole

Development (2021) 148, dev195347. doi:10.1242/dev.195347

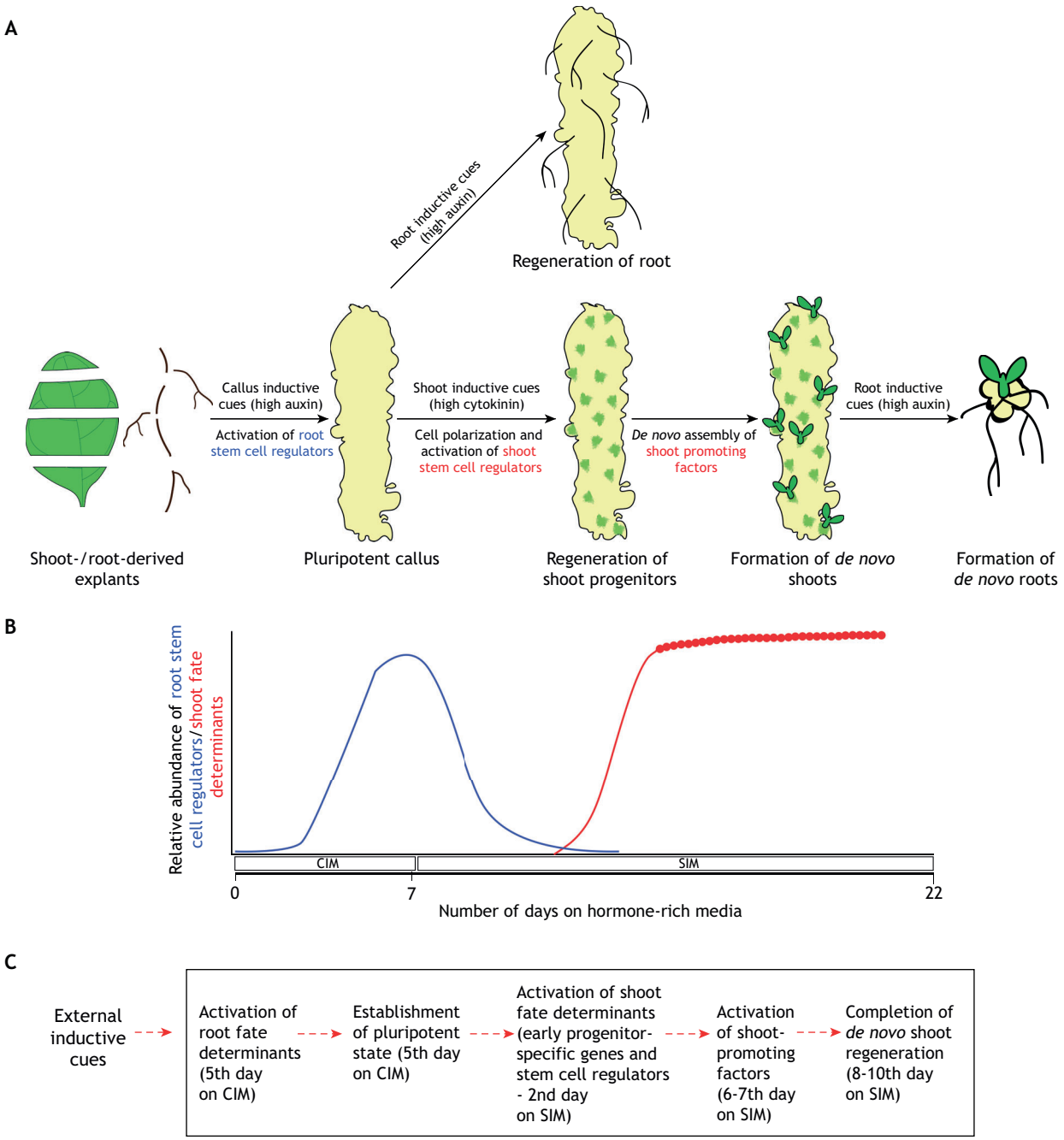
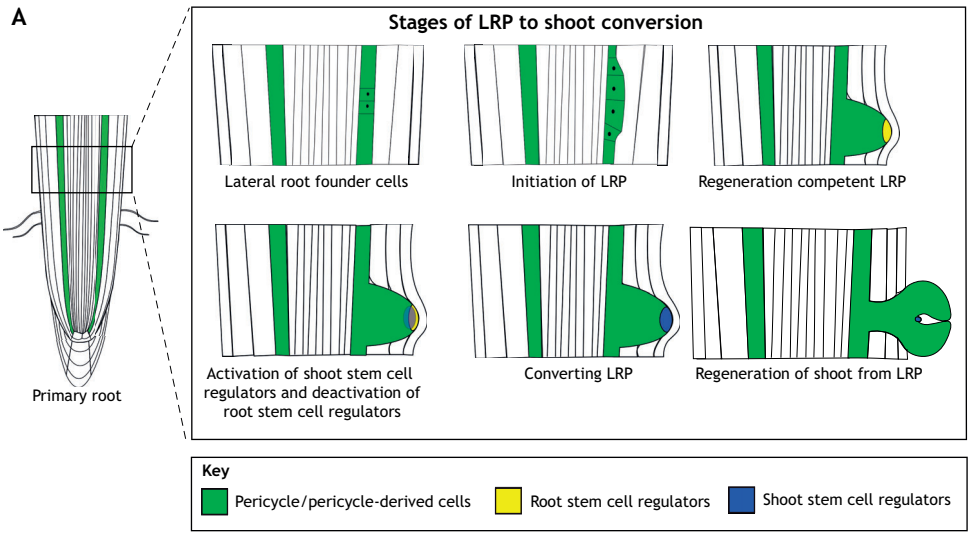


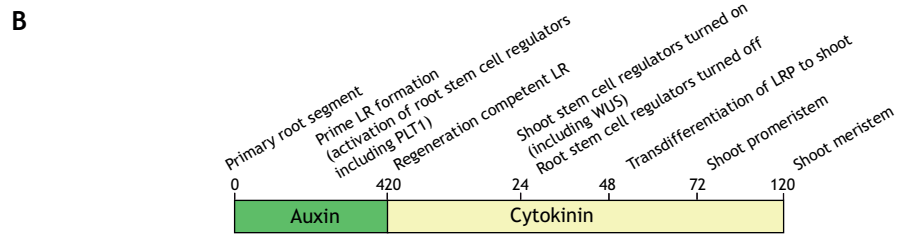
Fig. 2. Indirect tissue culture-induced regeneration. (A) Summary of the events that occur during tissue culture-mediated root (top) and shoot (bottom) regeneration. Explants of different developmental origins first form a pluripotent callus. They then undergo self-organization and, if cultured in the presence of shoot inductive cues, form shoot progenitor cells, ultimately culminating in the formation of a shoot (bottom). Note that not all shoot progenitors convert into shoots; if explants are cultured in root inductive cues, root formation occurs (top). (B) Graph depicting the relative abundance of root stem cell regulators and shoot fate determinants (*y*-axis) against the days (*x*-axis) of explant culture on callus induction medium (CIM) or shoot induction medium (SIM). Root stem cell regulators (blue) are transiently upregulated throughout the callus while it is incubated on CIM, ensuring acquisition of pluripotency, and are downregulated following transfer of the callus onto SIM. In response to shoot inductive cues, shoot fate determinants (red) get activated. Their expression peaks by the time the callus undergoes shoot progenitor regeneration and self-assembly (denoted by broad red dots), leading to the formation of an entire shoot *de novo*. (C) Regulatory module highlighting the sequential events occurring during regeneration of a shoot system *de novo*.

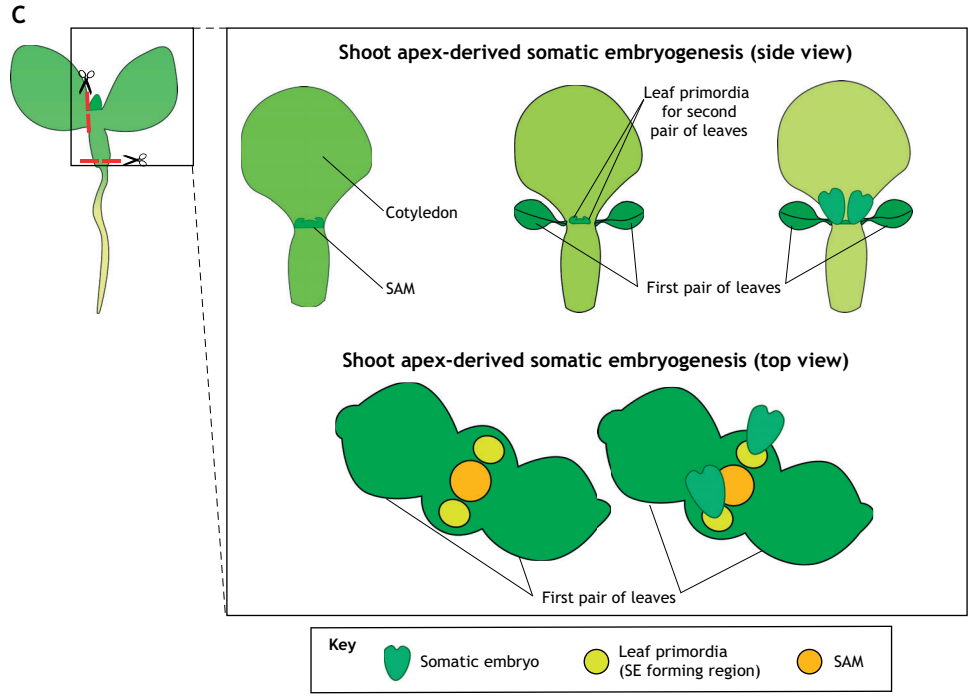
pericycle (XPP) cells that, during normal development, give rise to lateral roots (LRs) (Atta et al., 2009; Malamy and Benfey, 1997; Sugimoto et al., 2010). The molecular nature of this callus has been studied extensively to reveal the regulators controlling the formation of a callus and its acquisition of pluripotency.

During callus formation, one of the regulators of LR formation, ABERRANT LATERAL ROOT FORMATION 4 (ALF4), plays a crucial role (DiDonato et al., 2004). ALF4 modulates auxin signalling and is essential for LR initiation (Bagchi et al., 2018); it is this involvement in the auxin response that possibly makes ALF4 crucial for callus formation. Interestingly, the callus predominantly expresses root-specific genes (Sugimoto et al., 2010). Although callus formation necessitates activation of *ALF4*, pluripotency acquisition by the callus requires the recruitment of root stem cell regulators such

Development (2021) 148, dev195347. doi:10.1242/dev.195347







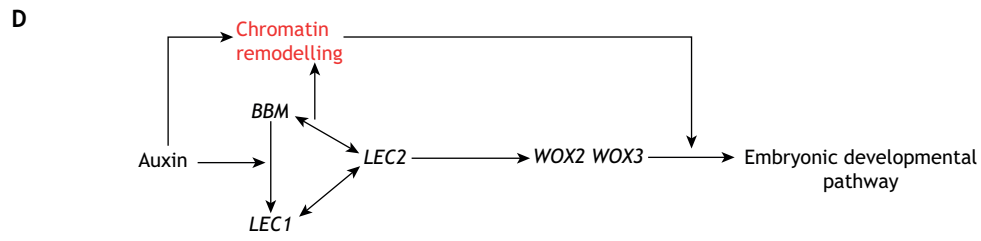


Fig. 3. Direct tissue culture-induced regeneration. (A) The stages involved in shoot regeneration via direct conversion of lateral root primordia (LRP). The primary root is primed to produce regeneration-competent LRP, which, when induced with shoot inductive cues, develop into a shoot. Note that the LRP arises from cells of the pericycle. By the time shoot stem cell regulators such as WUSCHEL (WUS) are activated, root stem cell regulators such as PLT1 are rapidly deactivated and become undetectable in the converting LRP. (B) Timeline of the events occurring during conversion (trans-differentiation) of an LRP to a shoot. During the first stage (0-42 h; green) the primary root is incubated on auxin-rich medium, after which stage (0-120 h; yellow) the auxin-primed root is incubated on cytokinin-rich medium. (C) Direct regeneration of somatic embryos from leaf primordia. The developing young leaf primordia of the second pair of leaves can be induced to form a somatic embryo. (D) Regulatory module controlling the activation of embryonic developmental pathways during somatic embryogenesis.

Development (2021) 148, dev195347. doi:10.1242/dev.195347

as PLETHORA (PLT)1, PLT2, SCARECROW (SCR) and WUSCHEL RELATED HOMEOBOX 5 (WOX5) (see Box 3 and Fig. 2B) (Kareem et al., 2015; Kim et al., 2018; Sugimoto et al., 2010). The onset of the expression of root stem cell regulators is under the control of several genetic and epigenetic regulators (Kareem et al., 2015; Kim et al., 2018).

Once a pluripotent callus has formed, its further fate in regeneration is decided by two major plant hormones: auxin and cytokinin. When supplemented with a higher auxin:cytokinin ratio the callus initiates root regeneration, whereas when supplemented with higher cytokinin:auxin it initiates shoot regeneration (Fig. 2A) (Skoog and Miller, 1957). Shoot regeneration is fine-tuned by two groups of regulators of cytokinin signalling, namely Type A *Arabidopsis* response regulators (ARRs), which are negative regulators, and Type B ARRs, which are positive regulators (for a review see To and Kieber, 2008). Shoot regeneration necessitates the activation of Type B ARRs and the removal of epigenetic repression marks from shoot stem cell regulators (Meng et al., 2017; Zhang et al., 2017). The resulting cytokinin signalling response activates shoot stem cell regulators and several shoot-promoting factors, thereby facilitating *de novo* shoot regeneration (Fig. 2B) (Atta et al., 2009; Iwase et al., 2017; Kareem et al., 2015). In the process, shoot initials called progenitors are formed before the formation of a functional shoot meristem. Although all cells of the callus are theoretically pluripotent, shoot progenitors do not initiate from all cells but rather arise stochastically from a few cells of the callus. They can be identified using fluorescent-labelled markers that mark the polar auxin transporter PINFORMED (PIN1) and the shoot stem cell regulator WUSCHEL (WUS) (see Box 3) (Gordon et al., 2007; Zhang et al., 2017). Further, not all the shoot progenitors can make it to completion of shoot regeneration, indicating that the callus is a highly heterogeneous mass of cells (Gordon et al., 2007; Lardon et al., 2020; Motte et al., 2014; Radhakrishnan et al., 2018).

In response to shoot inductive cues, root-specific genes that are instrumental in generating a pluripotent landscape get downregulated, with a concomitant increase in shoot-promoting factors, thereby facilitating the onset of shoot regeneration. The journey from acquisition of pluripotency to completion of shoot regeneration involves an interesting two-step molecular mechanism comprising three redundantly acting PLT genes, namely, *PLT3*, *PLT5* and *PLT7*. These PLT genes control lateral organ positioning during normal root and shoot development (Hofhuis et al., 2013; Prasad et al., 2011). In the first step, the PLT genes activate root stem cell regulators, making the callus competent to regenerate shoot progenitors. In the second step, which is dependent on the first step, shoot-promoting factors such as *CUP-SHAPED COTYLEDON 2* (*CUC2*) are activated by the PLT genes to accomplish regeneration of the complete shoot system (Kareem et al., 2015) (see Box 3; Fig. 2C). Acquisition of competency for shoot regeneration is thus uncoupled from the completion of shoot regeneration from a callus. This two-step mechanism operates in all explants irrespective of their developmental origin.

Tracking a battery of cell-fate determinants in response to shoot inductive cues in real-time has provided evidence that the initial assembly of the SAM *de novo* follows a path distinct from that used for SAM development during embryogenesis (Gordon et al., 2007; Kareem et al., 2015; Sugimoto et al., 2010). In the absence of embryonic positional cues, shoot regeneration is likely guided by an inherent property of regenerating cells to self-organize into a shoot (Gordon et al., 2007; Radhakrishnan et al., 2018). This self-organization is predominantly determined by two factors: acquired positional cues and the co-ordinated cellular and molecular interactions among regenerating progenitors. However, the molecular nature of callus heterogeneity and self-organization remains to be fully explored.

De novo shoot regeneration via trans-differentiation

Regeneration of a complete shoot system can also be achieved without an intermediary callus, whereby an LRP within a root explant is reprogrammed to generate a shoot directly, providing a classical example of trans-differentiation (see Glossary, Box 1) (Atta et al., 2009; Chatfield et al., 2013; Kareem et al., 2016; Rosspopoff et al., 2017). The transient expression of root stem cell regulators and an abundance of cytokinin are key determinants for shoot regeneration via trans-differentiation. Mutants such as *plt3*/*5*/*7*, which do not express root stem cell regulators, fail to undergo LRP trans-differentiation even in the presence of abundant external cytokinin (Kareem et al., 2015). Thus, the necessity of root stem cell regulators appears to be common for both callus-mediated shoot regeneration and trans-differentiation-based regeneration.

The cells within the quiescent centre (QC; see Glossary, Box 1) of LRP are receptive to both auxin and cytokinin during a narrow developmental window, and they assume distinct fates depending on the abundance of a particular hormone (Kareem et al., 2015, 2016; Rosspopoff et al., 2017) (Fig. 3A,B). Although auxin

Box 3. Key gene families involved in plant regeneration

PLETHORA (PLT) genes. PLTs belong to the AP2/ERF domain-containing family of plant-specific transcription factors (TFs). Root-expressed PLTs control root growth and LR emergence, whereas shoot-expressed PLTs regulate shoot apical meristem and lateral organ emergence. PLTs are also involved in numerous regenerative responses. PLT2 along with other redundant PLTs mediates root tip regeneration, whereas PLT3, PLT5 and PLT7 are essential for callus-mediated *de novo* shoot regeneration and trans-differentiation of a lateral root primordia into a shoot (Durgaprasad et al., 2019; Kareem et al., 2015). PLT3, PLT5 and PLT7 control shoot regeneration via a two-step mechanism, wherein they first activate the root stem cell regulators PLT1 and PLT2 to establish pluripotency and then activate the shoot-promoting factor CUC2 to accomplish shoot regeneration. The same PLTs promote vascular regeneration in growing aerial organs by activating CUC2, and not through root stem cell regulators.

WUSCHEL RELATED HOMEOBOX (WOX) genes. WOX proteins belong to a family of plant-specific homeodomain TFs. They mark region-specific cell fate decisions during early embryogenesis in *Arabidopsis* by virtue of their expression dynamics (Haecker et al., 2004). Besides their role in embryonic patterning, WOX genes such as *WOX11*, *WOX12*, *WOX5* and *WOX7* mediate adventitious root regeneration during *de novo* root regeneration (DNRR) (Liu et al., 2014).

LATERAL ORGAN BOUNDARY DOMAIN (LBD) genes. LBDs belongs to a family of plant-specific TFs with roles in shaping plant architecture. During normal *Arabidopsis* development, LBDs mediate auxin-induced lateral root formation. During regeneration, *LBD16* and *LBD29* are upregulated by WOX11 and WOX12 to mediate DNRR from the leaf (Hu and Xu, 2016; Liu et al., 2014). In addition, *LBD16*, *LBD17*, *LBD18* and *LBD29* are induced by auxin to promote callus formation in *Arabidopsis* (Fan et al., 2012; Xu et al., 2018).

***WOUND INDUCED DEDIFFERENTIATION 1* (*WIND1*) gene.** WIND1 belongs to the AP2/ERF family of TFs. Although its role during normal development in *Arabidopsis* remains unknown, it promotes callus formation in response to injury and promotes *de novo* shoot regeneration by activating *ENHANCER OF SHOOT REGENERATION* (*ESR1*) (Iwase et al., 2011, 2017).

***ETHYLENE RESPONSE FACTOR* (*ERF115*) gene.** ERF115 belongs to the Ethylene Response family of plant TFs. During normal development, it mediates root stem cell niche maintenance by acting as a rate-limiting factor for quiescent centre division (Heyman et al., 2013). Upon injury, it regulates root tip regeneration as well as cell type-specific regeneration (Zhou et al., 2019).

treatment induces LRs, cytokinin treatment of the root explants precisely during this developmental window ensures trans-differentiation to a shoot. This trans-differentiation is achieved by rapidly downregulating root stem cell regulators and turning on shoot stem cell regulators in QC cells (Fig. 3B) (Rosspopoff et al., 2017). Thus, the QC cells in LRP are highly plastic during this narrow window. This plasticity can be exploited by repeatedly switching the explants between cytokinin and auxin treatments, thereby switching between shoot and root fates, respectively (Rosspopoff et al., 2017). The multiple-rounds of cell fate switches are most likely attributed to a transient mixed-cell fate identity during the brief period of LRP development.

Regeneration of complete plant system via somatic embryogenesis

In addition to generating shoots and roots, explants can give rise to an entire embryo via the process of somatic embryogenesis. In this process, the somatic cells of either zygotic tissue or young plant tissue can be induced (often with external synthetic auxin higher than that required for *de novo* shoot regeneration) to form somatic embryos. Immature zygotic tissues such as the suspensor (see Glossary, Box 1) can be cultured *in vitro* to generate somatic embryos (Gaj, 2001; Radoeva et al., 2020 preprint; Wu et al., 1992). Similarly, somatic embryos can be induced from adult somatic tissues such as leaf protoplasts, floral buds and the shoot apex. A well-thought notion was that the meristem from the shoot apex was the source for somatic embryos (Luo and Koop, 1997; Ikeda-Iwai et al., 2003). However, careful analysis through RNA-seq and live-imaging of numerous fate determinant markers revealed that somatic embryos are induced directly from the base of young leaf primordia of the shoot apex and not the SAM (Fig. 3C) (Kadokura et al., 2018). Here, the leaf primordia cells are competent to produce somatic embryos only during a narrow developmental window, and leaf primordia cells outside this developmental stage die or resort to callusing (Kadokura et al., 2018). Upon somatic embryo induction, the young leaf primordia cells start expressing embryogenic root as well as SAM identity genes and thus acquire a mixed cell-fate identity. Such a molecular environment in the leaf primordia cells is likely to facilitate the switch to an embryonic fate (Kadokura et al., 2018).

A closer examination at the chromatin level has revealed that somatic embryogenesis involves highly organized chromatin remodelling. Using a combination of assays for transposase-accessible chromatin sequencing (ATAC-seq), RNA-seq and chromatin immunoprecipitation (ChIP)-seq, it was found that auxin modulates the chromatin accessibility and thereby the dynamics of global gene expression in a developmental stage-specific manner during somatic embryogenesis (Fig. 3D) (Wang et al., 2020b). Numerous TFs controlling somatic embryogenesis have also been identified (Horstman et al., 2017). For example, ectopic overexpression of a member of the AP2/ERF family of TFs, BABY BOOM (BBM; also known as PLETHORA4), which acts as a root stem cell regulator during normal development, induces somatic embryos (Galinha et al., 2007; Boutilier et al., 2002). BBM has been shown to activate early embryogenic genes such as *WOX2* and *WOX3* via the TFs LEAFY COTYLEDON 1/2 (LEC1/2), wherein BBM binds to the promoter of *LEC1* and *LEC2* to transcriptionally upregulate their expression (Fig. 3D) (Horstman et al., 2017; Wang et al., 2020b). In addition to genetic control, these genes are epigenetically regulated. Concurrently, it was shown that removal of epigenetic repression marks on *LEC2* favours somatic embryogenesis from root hairs (Ikeuchi et al., 2015). Thus, SAM formation during somatic embryogenesis is likely to be pre-determined by embryonic positional cues, unlike the callus-mediated shoot regeneration that occurs in the absence of such positional cues.

Mechanical injury-induced regeneration

Wound repair and regeneration in underground organs

Plants exhibit numerous mechanical injury-induced regenerative responses throughout their body parts during normal growth. Growing roots, for instance are prone to injury by biotic and abiotic factors, often leading to loss of root cells or even the root tip. Laser ablation, cytotoxic drug treatment, the resection of *Arabidopsis* root tips, as well as simulations mimicking ablation and resection have been used to study the mechanisms underlying wound-induced regeneration (Fulcher and Sablowski, 2009; Grieneisen et al., 2007; Hong et al., 2017; Sena et al., 2009; van den Berg et al., 1995, 1997; Zhang et al., 2016).

The root meristem undergoes small-scale local regeneration responses in the form of restoration of individual damaged cells, including the QC (Fig. 4). The targeted laser ablation of QC cells disrupts auxin flow and rapidly upregulates the auxin response a few cell layers above the damaged QC. This proximal shift in auxin responses allow vascular cells present 1-2 cell layers above the ablated QC to acquire a stem cell fate and get respecified into new QC cells, whereas cell layers between the ablated and regenerated QC get respecified into columella cells (van den Berg et al., 1995; Xu et al., 2006) (Fig. 4E). Laser ablation of a root meristem cell other than a QC cell stimulates its inner adjacent cell to acquire a stem cell fate and undergo periclinal division. Fate respecification of the resulting daughter cell assures restoration of the lost cell; this phenomenon stays true to all major root meristem cell types (Marhava et al., 2019; van den Berg et al., 1995) (Fig. 4E). Positional cues have been implicated in the replacement of lost cells, and turgor pressure shock from dead cells has been suggested to influence the expansion of neighbouring cells (Hoermayer et al., 2020; Marhava et al., 2019; van den Berg et al., 1995). However, why division and respecification is exclusive to the cell inner to damaged ones remains elusive. It is known that growing roots experience intrinsic axial and radial growth pressure, the effect of which presumably differs between inner and outer cell files (Clark et al., 2003). This differential growth pressure possibly renders the inner cell to compensate for loss of its outer cell.

Besides small-scale regenerative responses, the root meristem exhibits comparatively large-scale regeneration, for example the restoration of excised root tips. A combination of lineage tracing, single-cell RNA-seq and live imaging has unveiled that *Arabidopsis* root tip restoration upon resection follows an embryonic pathway (Efroni et al., 2016). Here, proliferating cells at the cut end experience a brief overlap in auxin and cytokinin expression domains akin to that observed in the embryo. This similarity in hormonal dynamics triggers the expression of early embryonic genes such as *MONOPTEROS* and guides the subsequent activation of root stem cells, which leads to complete root tip restoration (Efroni et al., 2016) (Fig. 4A). Interestingly, root tip excisions close to the distal end of the root meristem allow root tip restoration, whereas proximal excisions within the root meristem fail to do so. Thus, there exists a boundary in the root meristem beyond which root tip fails to regenerate. The dosage of the root stem cell regulator PLT2 (see Box 3), which is expressed in a gradient along the root meristem, instructs this boundary (Fig. 4C). The mitotic dilution and cell-to-cell movement of PLT2 facilitates its graded distribution from the distal to the proximal end of the root meristem; high levels of PLT2 activate stem cells, low levels

Development (2021) 148, dev195347. doi:10.1242/dev.195347

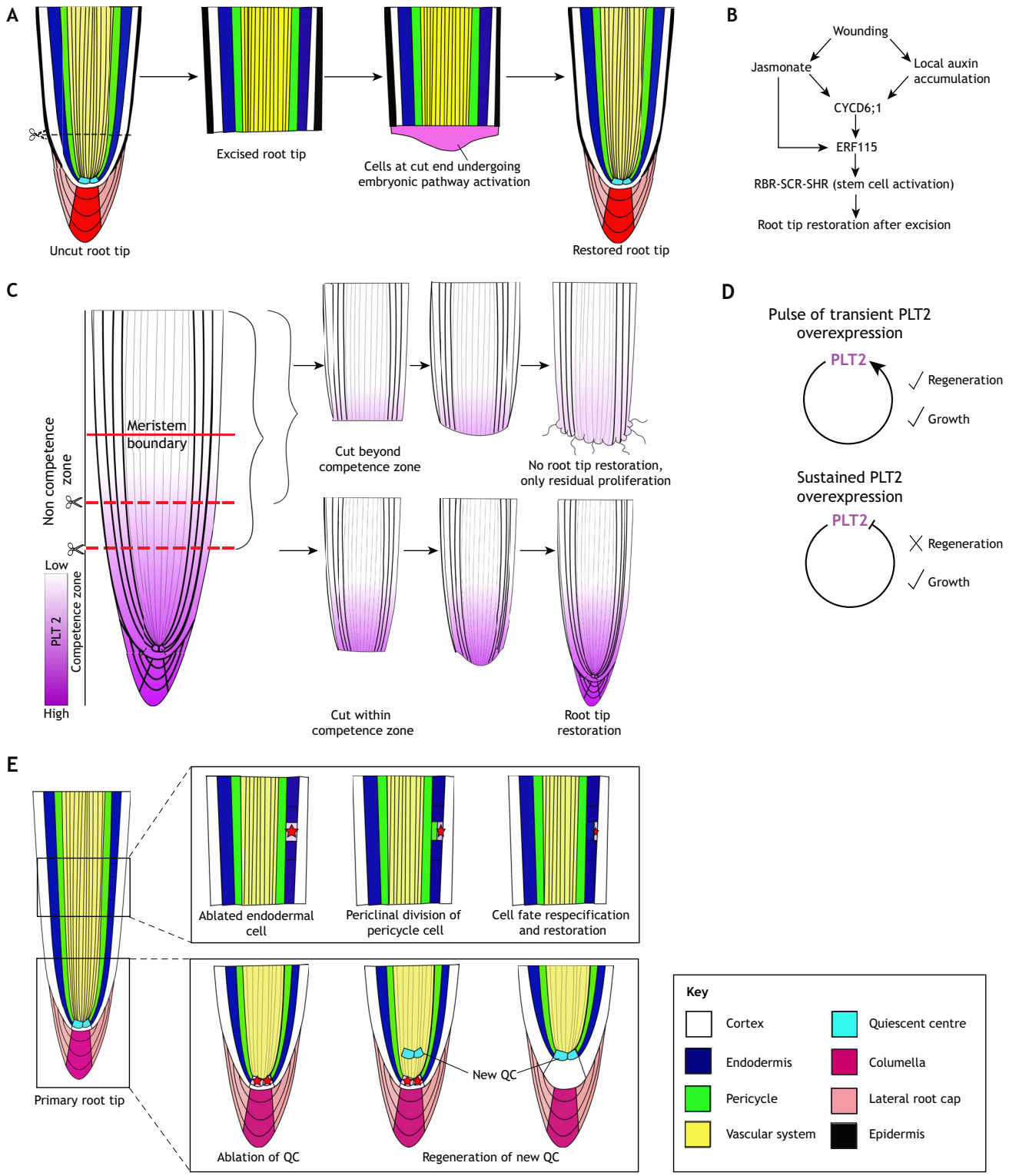


Fig. 4. Regeneration of the root tip and its cells. (A) Upon root tip excision, uninjured endodermal/stelar cells at the cut end proliferate and undergo transient activation of embryonic signalling pathways to restore the missing root tip. (B) Regulatory module showing how wound-induced jasmonic acid (JA) and auxin accumulate to trigger stem cell activation (i.e. the RBR-SCR-SHR protein network) via the ethylene response gene, ERF115, and a regulator of cell cycle progression, CYCLIN D6 (CYCD6;1). This ultimately enables root tip restoration following excision and stem cell niche regeneration after quiescent centre (QC) ablation. (C) The concentration of PLETHORA 2 (PLT2), a transcription factor that is expressed in a gradient along the root meristem, instructs the boundary for root tip restoration. The root tip is restored when excision is within the region of meristem expressing high levels of PLT2 (top) but restoration fails when the tip is excised in a region expressing low PLT2 levels (bottom). (D) Schematic depicting the importance of the PLT2-autoregulatory loop in regeneration and how it distinguishes growth of the root meristem from its regeneration potential. (E) Upon ablation of an endodermal cell (top inset), a pericycle cell from the inner cell file undergoes periclinal division and the resulting daughter cell respecifies its fate to replace the lost endodermal cell. Targeted laser ablation of the QC (bottom inset) results in its regeneration two cell layers above the initial QC, while the cell layers below the new QC become specified into columella cells.

promote cell division and even lower levels are required for differentiation (Mähönen et al., 2014). *PLT2*, together with other redundant PLT genes, is essential for root tip regeneration and acts in a positive auto-regulatory loop to grant regeneration potential (Fig. 4D). This auto-regulatory loop collapses upon sustained overexpression of *PLT2*. In such conditions, the root meristem of uninjured roots grows longer than normal, but its root tip restoration ability ceases and gets substituted with mere residual cell proliferation (Fig. 4D). Thus, the threshold-sensitive auto-regulatory loop distinguishes the growth of the organ from its regeneration potential (Durgaprasad et al., 2019).

Auxin biosynthesis and its polar transport are pivotal for organ formation during normal development as well as for root tip regeneration (reviewed by Shanmukhan et al., 2020b). The extent of injury, i.e. large-scale or small-scale, acts as the decisive factor for the magnitude of auxin required for cell or organ regeneration. In the case of minor injuries to the root meristem, such as targeted cell damage, ongoing auxin production and its accumulation due to impeded transport is sufficient to stimulate small-scale regeneration (Canher et al., 2020). However, large-scale regeneration such as root tip restoration necessitates further upregulated auxin production (Matosevich et al., 2020). Several root fate determinants and regulators of root stem cell maintenance have been implicated in regeneration, although how these regulators function in conjunction with auxin needs to be studied (Durgaprasad et al., 2019; Efroni et al., 2016; Marhava et al., 2019; Matosevich et al., 2020; Xu et al., 2006). An interesting possibility is that the fate determinants and auxin act in a regulatory loop to control the regeneration of specific cell types or organs.

Auxin, along with another plant hormone jasmonic acid (JA), triggers a common signalling network to accomplish regeneration following root tip excision as well as ablation. The two hormones accumulate locally upon injury and act synergistically to trigger expression of the stress response protein ETHYLENE RESPONSE FACTOR 115 (ERF115) and a cell cycle regulator CYCLIND6;1 (CYCD6;1), which in turn activates the RETINOBLASTOMA-RELATED (RBR)-SCR-SHORTROOT (SHR) protein network (Zhou et al., 2019) (Fig. 4B). Root tip restoration necessitates reactivation of the SCN, which in turn is regulated by the RBR-SCR-SHR protein network (Zhou et al., 2019). The variety of regenerative responses in the *Arabidopsis* root meristem discussed here relies on regulators that are required not only for regeneration, but also for root meristem development, thereby providing a nice example of the interplay between regenerative mechanisms and those governing development (Cruz-Ramírez et al., 2012, 2013; Durgaprasad et al., 2019; Efroni et al., 2016; Galinha et al., 2007; Hardtke and Berleth, 1998; Mähönen et al., 2014; Zhou et al., 2019).

Auxin also plays a role in root regeneration following abiotic stress, for example following exposure to cold temperatures. Here, cells of the SCN – including QC cells – are protected by sacrificing columella stem cell daughters (CSCDs). Similar to the anatomical block in auxin transport by ablated QC cells, which leads to regeneration of new QC, the dead CSCD cells block auxin transport resulting in elevated auxin levels in the QC, which in turn prolongs the existing QC instead of regenerating a new one (Hong et al., 2017). Other multi-functional plant growth hormones such as ethylene, mostly known for its role in fruit ripening and senescence, are also implicated in the regenerative response to stress. For example, biotic stress such as a nematode attack in the root meristem (which can be considered analogous to laser ablation) stimulates ethylene signalling in addition to JA signalling. Whereas ethylene elicits a local defence response against the nematode, JA encourages its reproductive success besides promoting root regeneration (Zhou et al., 2019). These two pathways, which are seemingly antagonistic to each other, are likely to promote plant vitality through defence and regeneration. Studies addressing the fundamental mechanisms of root regeneration and their physiological relevance in *Arabidopsis* therefore open up the possibility of carrying out similar such studies in other plant species. This will also allow parallels between parasite-induced responses and mechanical-injury induced responses to be drawn.

Wound repair and vascular regeneration in aerial organs

Much like underground parts, aerial parts of the plant are injured frequently and elicit varying regenerative responses. Injuries mimicking natural mechanical damages to growing *Arabidopsis* aerial organs, such as vascular injury and surface abrasion, have allowed the molecular mechanisms underlying their regeneration to be probed. Both surface abrasion and deep incisions in the stem that slice through the vasculature trigger cell proliferation; however, subsequent vascular regeneration and reunion is exclusive to the latter (Fig. 5A) (Asahina et al., 2011; Flaishman et al., 2003; Radhakrishnan et al., 2020a). During vascular reconnection, the regenerating vasculature bypasses the wound and this phenomenon is observed following stem as well as leaf mid-vein incisions (Fig. 5A,B) (Flaishman et al., 2003; Radhakrishnan et al., 2020a). The presumption that ground tissue can be induced to form vascular cells during vascular regeneration in the presence of sufficient auxin flux led to the auxin canalization hypothesis (Sachs, 1969, 1981, 1991). Auxin canalisation is promoted by polar localization of phosphorylated PIN1 (an auxin efflux carrier) on the plasma membrane during vascular regeneration in the *Arabidopsis* stem (Hajný et al., 2020). PIN1 fails to polarise in the absence of a TF-based regulatory axis (discussed below) leading to failure of vascular regeneration in growing leaves and further substantiating the canalization model for vascular regeneration (Radhakrishnan et al., 2020a).

Vascular regeneration in growing *Arabidopsis* aerial organs requires the activity of *PLT3*/*5*/*7* and *AINTEGUMENTA* genes. However, in this context, PLT3/5/7 do not activate root stem cell regulators like they do in tissue culture-mediated regeneration, in which their activation is indispensable. Instead, PLT genes directly bind to the promoter of another TF gene, *CUC2*, in response to injury. The *PLT-CUC2* regulatory axis acts in a coherent feed-forward loop to upregulate local auxin production, which in turn is essential for vascular regeneration and for guiding the newly formed vein along a polarized path for reunion with its parental strands (Fig. 5C) (Radhakrishnan et al., 2020a). Here, the high auxin signalling is likely to serve as a cue for ground tissue cells to undergo vascular cell activation that appears to steer the path of the regenerating vein. The likely termination of this feed-forward loop could thus be causal for the cessation of vein regeneration upon complete reconnection between parental strands. Nevertheless, how the regenerating vein follows a path and recognizes its parental strands hitherto remains unknown. Tracking the path of this injury-induced newly formed vein offers an excellent system to study the recognition, communication and reunion between physically disconnected tissues. In addition, vein regeneration efficiency declines progressively with increasing seedling age, with the increase in wound size, and as the position of injury shifts farther from the leaf-base (Radhakrishnan et al., 2020a,b preprint). Although the exact mechanism underlying the age- and position-dependent efficiency of this regeneration is unknown, it could

Development (2021) 148, dev195347. doi:10.1242/dev.195347

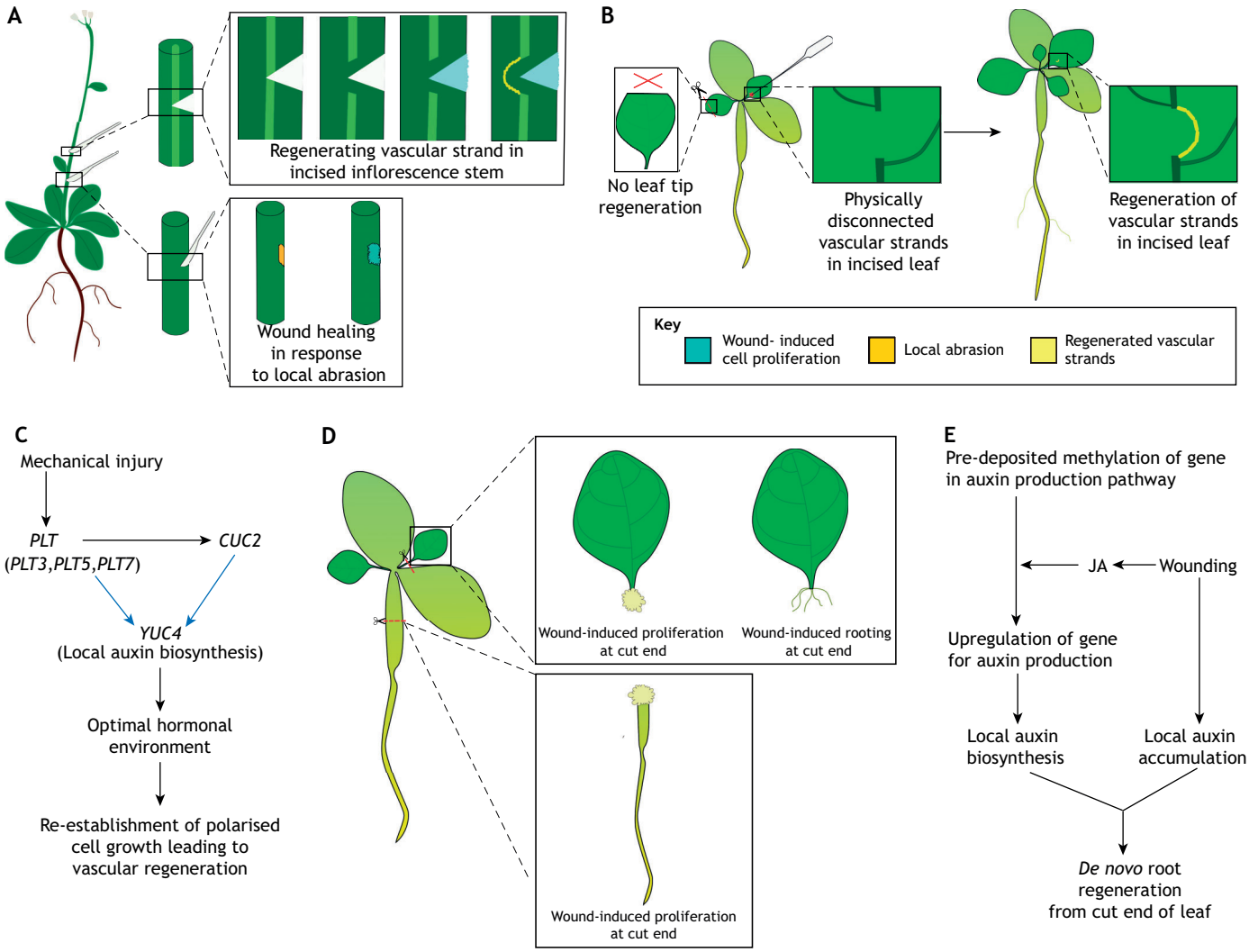


Fig. 5. Regenerative responses in *Arabidopsis* aerial organs. (A) Incision of the inflorescence stem (top inset) disrupts vascular tissue continuity and induces cell proliferation to seal the gap between the disconnected tissues. Subsequently, vascular continuity is reinstated by the vascular stands regenerating around the wounded area. By contrast, surface abrasion of the inflorescence stem (bottom inset) induces only local cell proliferation to heal the injury. (B) Excision of the distal end of a growing leaf (left inset) does not initiate regeneration to replace the lost part. By contrast, incision of the leaf mid vein (right inset) disrupts vascular continuity. The regenerating vasculature circumvents the wounded area to re-establish vascular continuity in the growing leaf. (C) Schematic depicting the *PLT-CUC2* regulatory module involved in vascular regeneration in aerial organs. (D) A regenerative response occurs (in the form of wound-induced callus formation) from the cut end of a detached leaf when the cut end does not touch the media (top left). However, when the cut end of the detached leaf is in contact with the nutrient-rich media, wound-induced rooting occurs (top right). Wound-induced callusing also occurs at the cut end of detached hypocotyls when placed onto nutrient-rich media (bottom). (E) Regulatory module showing the mechanism of *de novo* root regeneration from the cut end of a detached leaf.

likely be attributed to modulation of the *PLT-CUC2* regulatory axis. Strikingly, vein development and patterning in *plt* or *cuc2* mutants remain unaffected during normal growth (Radhakrishnan et al., 2020a). The injury-induced *PLT-CUC2* regulatory axis therefore distinguishes vein regeneration from its formation during normal development.

Regenerative responses at the cut ends of detached organs: insights into plasticity and ageing

A feature unique to plant regeneration is the ability of cells at the cut end of a detached organ to adopt an identity different from their pre-existing one. For example, when cultured on hormone-free medium, an excised *Arabidopsis* leaf elicits one of the two following regenerative responses from its cut end: the formation of a new organ, i.e. *de novo* root regeneration (DNRR), or local healing in the form of callus formation (Fig. 5D) (Chen et al., 2014; reviewed by Ikeuchi et al., 2016; Iwase et al., 2011, 2017).

During DNRR, the rapid yet transient accumulation of wound-induced JA, in conjunction with pre-deposited methylation on JA responsive genes, upregulates auxin production at the site of injury, which in turn facilitates DNRR (Fig. 5E) (Zhang et al., 2019). The auxin maxima precedes vascular cell proliferation and the induction of WOX genes, namely *WOX11* and *WOX12* near the cut end of the leaf, eventually resulting in the formation of a small mass of cells with rooting competence (Bustillo-Avendaño et al., 2018; Zhang et al., 2019). Such cells undergo a two-step cell fate transition to initiate the formation of a root primordia: *WOX11*/*12* along with LBD genes (see Box 3) regulates the transition of rooting competent cells into root founder cells, marking the first step, whereas *WOX5*/*7* regulate the transition of root founder cells into a root primordium, marking the second step (Hu and Xu, 2016; Liu et al., 2014). The

subsequent formation and maintenance of the root primordium by root stem cell regulators results in activation of the root meristem and, ultimately, root regeneration (Bustillo-Avendaño et al., 2018). In addition to these TFs, PLT proteins are essential for DNRR (Shanmukhan et al., 2020a preprint). Moreover, a decline in this form of root regeneration has been observed from aged leaf explants. Although the implications of ageing on many aspects of development have been well documented in both plants and animals, this area remains less explored with respect to regeneration. At least in DNRR, the diminished regeneration potential of older *Arabidopsis* leaf explants is partly attributed to the age-induced accumulation of ethylene, which inhibits WOX genes and, consequently, DNRR (Li et al., 2020).

Aside from DNRR, the cut end of detached leaf exhibits a distinct wound-induced callusing response. Here, the activation of an early wound response gene, *WOUND INDUCED DEDIFFERENTIATION 1* (*WIND1*; see Box 3), causes cells at the cut end to undergo dedifferentiation (see Glossary, Box 1) and subsequent cell proliferation to form a callus (Fig. 5E) (Iwase et al., 2011). Unlike the hormone-induced callus that arises in tissue culture, the wound-induced callus does not predominantly express root fate determinants (Iwase et al., 2011). In addition, the hormone-induced callus arises from XPP cells and spreads throughout the explant, whereas the injury-induced callus forms from and is confined to the site of injury (Atta et al., 2009). This is because the regeneration machinery distinguishes the uninjured tissues from the injured ones by tight transcriptional control of respecification genes. Such control is mediated partly by epigenetic mechanisms, whereby modifications to histone N-terminal tails regulate transcription of the associated genes. For example, the majority of genes that are rapidly induced by wounding, including *WIND1*, are marked by histone acetylation immediately before/after wounding, thereby allowing them to promote callus formation only at the site of injury. A correlation between the expression of wound responsive genes and their epigenetic modifications has recently been revealed using ChIP-seq and RNA-seq (Rymen et al., 2019).

The deciding factor for either of the two responses – DNRR or callus formation – at the cut end of a detached leaf remains unclear. DNRR results when the cut end touches the media, as opposed to callus formation, which occurs when the cut end fails to touch the media (Fig. 5D) (Iwase et al., 2017; Shanmukhan et al., 2020a preprint). As an increase in local auxin production is essential for both these responses, what distinguishes DNRR from callus formation is likely a touch-dependent mechanical cue (Iwase et al., 2017; Shanmukhan et al., 2020a preprint; Zhang et al., 2019). Among the several possible mechanical cues, osmotic stress has been implicated in the regeneration of root meristem cells upon targeted cell ablation (Hoermayer et al., 2020). However, the mechanisms underlying touch-driven mechano-sensing in regeneration remain largely unknown. Presumably, in DNRR at least, contact of the cut end with media activates touch-dependent mechano-sensors, and the resulting signal transduction in conjunction with local auxin accumulation likely promotes the production of rooting-competent cells (Shanmukhan et al., 2020 preprint).

Grafting: fusion between two body parts

The age-old agricultural technique by which two different body parts originating from two plants of the same or different species are joined to give rise to a chimera is called grafting. The parts that form the prospective shoot and root systems in a graft are called the scion and rootstock, respectively. Initially grafting was practised to combine aesthetically and agronomically desirable characteristics from two plants, such as disease resistance, increased productivity and early flowering (Goldschmidt, 2014). However, grafting in *Arabidopsis* has now become more relevant for addressing fundamental questions concerning the long-range transport of proteins, hormones, RNAs and secondary metabolites. Importantly, although it is a type of mechanical injury-induced regeneration, grafting by itself is a distinctive phenomenon and, as such, can provide insights into the molecular mechanisms involved in regeneration. Refined techniques have allowed micrografting of hypocotyls, leaves, and cotyledons in *Arabidopsis* (Bartusch et al., 2020; Huang and Yu, 2015; Nisar et al., 2012; Yoo et al., 2013). Such studies have unveiled the sequential events involved in grafting, from recognition of disrupted tissue continuity to successful graft formation.

Micrografting of *Arabidopsis* hypocotyls has revealed that, after cutting, auxin and starch accumulate above the cut and their asymmetric distribution on either side of the cut indicates a disruption in tissue continuity (Melnyk et al., 2018). In order to re-establish tissue continuity, recognition and adhesion between the two opposing body parts is imperative. Adhesion during grafting is facilitated by a cell wall remodelling enzyme, which often functions when the opposing parts are recognized as compatible (Notaguchi et al., 2020 preprint). The cues that trigger the recognition phenomenon are less known. However, there is evidence to suggest that the two opposing tissues have been recognized by each other. For example, the activation of *HIGH CAMBIAL ACTIVITY 2* (*HCA2*), an auxin response gene that is required for phloem reconnection, exclusively in the grafted bottom suggests that recognition has occurred. Neither the unattached bottom nor the intact hypocotyls express *HCA2*, suggesting that it is uniquely activated by grafting and highlighting that phloem reconnection is crucial to initiate the re-establishment of tissue continuity (Matsuoka et al., 2016; Melnyk et al., 2015, 2018). Another regulator of phloem reconnection is the lateral root formation gene *ALF4*, activity of which is required below the graft junction (DiDonato et al., 2004; Melnyk et al., 2015; Sugimoto et al., 2010). Sealing of the wound gap and subsequent vascular regeneration are common events in both partially incised stems of the same plant as well as in grafting between two plants, but what distinguishes grafting is the recognition upon tissue attachment (Fig. 6A,B) (Melnyk et al., 2018; Radhakrishnan et al., 2020a).

Refined techniques have been developed over the last decade to detect, monitor and quantify the movement of molecules across the graft junction. For example, the application of carboxyfluorescein diacetate (a membrane permeable fluorescent dye) to the scion and rootstock can be used to assay phloem and xylem connectivity, respectively (Melnyk et al., 2015). In addition, live imaging using confocal microscopy, qRT-PCR and RNA-seq of transgenics and mutants has yielded reliable data regarding long-range transport of mobile molecules in *Arabidopsis* (Melnyk et al., 2015, 2018; Turnbull, 2010). Although challenging, understanding the role of these long-range mobile molecules for plant regeneration will contribute significantly to future studies in the field.

Conclusions and perspectives

The advances in tools and resources available for *Arabidopsis* research have begun to provide deeper insights into the journey of plant regeneration and offer a promising direction for future studies in the area of cellular reprogramming and developmental plasticity. Auxin plays a crucial part in several cellular processes including cell

Development (2021) 148, dev195347. doi:10.1242/dev.195347

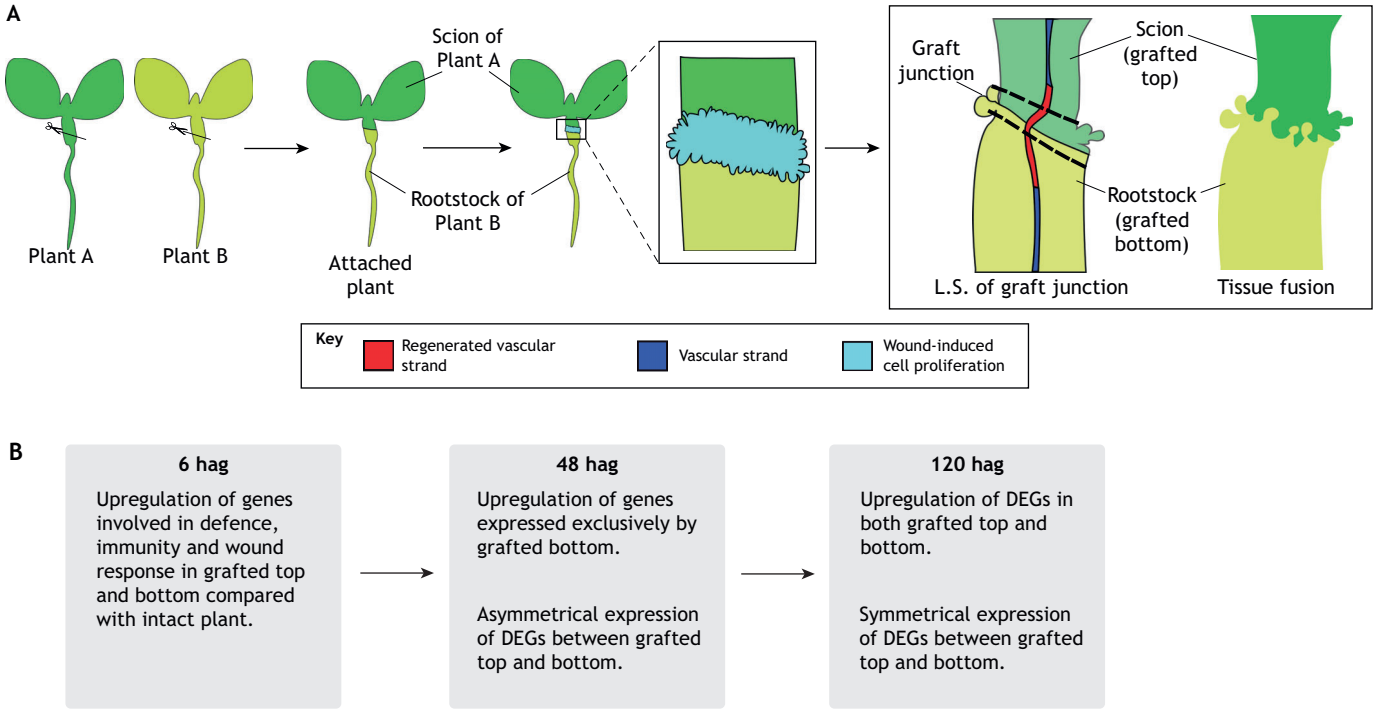


Fig. 6. Grafting between two plant parts. (A) The scion from plant A and the rootstock from plant B are attached together, resulting in wound-induced cell proliferation in the form of a callus (see inset). This seals the gap at the attachment plane and forms a graft junction (indicated by dashed lines). Subsequently, cells from both opposing parts interdigitate, ultimately leading to tissue fusion and re-establishment of vascular continuity. Note that the epidermal cells of a grafted top and bottom expand at the graft junction. (B) Timeline over which differentially expressed genes (DEGs) shift from being asymmetrically to symmetrically distributed between the grafted top and bottom. Note that DEGs are genes that are differentially expressed in grafted seedlings compared with intact seedlings. hag, hours after grafting; L.S., longitudinal section.

fate transitions during both development and regeneration, acting via global genetic regulation and epigenetic modifications, including chromatin remodelling. It functions in a concentration-dependent manner to yield a particular developmental outcome. As such, each regenerative response may depend on a specific auxin level. How a specific level of auxin is assigned to, and controls, a particular regenerative response as well as normal development can likely be attributed to: (1) context-dependent factors; (2) the magnitude of auxin production; (3) the source of auxin production; (4) its diffusion; (5) its polar auxin transport which generate a flux.

The generation of specific auxin levels during normal development has been explained *in silico* using two key models: the reaction-diffusion (RD) model and the flux-based model (Cruz-Ramírez et al., 2012; Grieneisen et al., 2007; Mündermann et al., 2005; Sampathkumar et al., 2014; Smith et al., 2006). The flux-based auxin canalization model elegantly explains gradient-driven self-organizing patterning (Bennett et al., 2014; Sachs, 1969, 1981, 1991). However, the model fails to sustain itself after a temporal perturbation and a major limitation is to test the model experimentally, particularly to measure flux (van Berkel et al., 2013). As such both the RD and flux models are likely required to explain the complex cell-cell interactions, and thereby the emergence of pattern, during regeneration. The expanse of data derived from experimental studies in *Arabidopsis* can now be used to examine the possibility of a switch between the two models, and how such a switch occurs during different regenerative responses.

Plants must be equipped to ensure the correct replacement of an organ or tissue lost in injury. As an immediate response to injury, cells at the wound vicinity experience degrees of variation with respect to noise in gene expression, cell polarity and cell division state, the cumulative effect of which is harnessed for the emergence of a particular pattern. However, not all such variations will be reflected in cellular behaviour. Thus, it will be challenging to tease out only the meaningful variations to interpret cellular heterogeneity. Although it will be daunting, finding the meaningful variations and precisely quantifying them will be key to gaining deeper insights into cellular reprogramming and fate transitions during regeneration. Moreover, when plants encounter injuries in varying conditions of nutrient availability and environmental stimuli, such as electrical stimuli, nitric oxide and physical contact, they are likely to tweak their innate wound repair mechanism in response to these conditions (Cervantes-Pérez et al., 2020; Kral et al., 2016; Shanmukhan et al., 2020a preprint). The challenge will be to test how fluctuating environmental conditions modulate regeneration-specific regulatory frameworks, and to understand their physiological relevance. Such a study already carried out in the *Arabidopsis* root tip (Marhavý et al., 2019) offers encouragement to conduct the same study in other plant parts and in other species in order to better understand how plants sense and respond to injury.

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Competing interests
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