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Review article

Wound signaling of regenerative cell reprogramming

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ABSTRACT

Plants are sessile organisms that must deal with various threats resulting in tissue damage, such as herbivore feeding, and physical wounding by wind, snow or crushing by animals. During wound healing, phytohormone crosstalk orchestrates cellular regeneration through the establishment of tissue-specific asymmetries. In turn, hormone-regulated transcription factors and their downstream targets coordinate cellular responses, including dedifferentiation, cell cycle reactivation and vascular regeneration. By comparing different examples of wound-induced tissue regeneration in the model plant *Arabidopsis thaliana*, a number of key regulators of developmental plasticity of plant cells have been identified. We present the relevance of these findings and of the dynamic establishment of differential auxin gradients for cell reprogramming after wounding.

Contents

1. Introduction

Regeneration of complex structures after an injury in multicellular organisms requires the initiation of a genetically-encoded developmental program for wound healing, programmed cell death, cell dedifferentiation, cell proliferation and new pattern formation [1]. Local responses at the site of the wound have important roles in the initiation of regenerative processes [2]. New organ regeneration in some metazoans, such as salamanders and newts, depends on the production of a mass of undifferentiated and proliferating cells, the blastema, which later becomes re-specified [3]. This type of regeneration is called epimorphosis. The blastemal cells arise either by cell proliferation of resident stem cells or by dedifferentiation towards stem cell-like precursors. In contrast to most animals, plants cells were thought to be totipotent, as mature cells cultured *in vitro* with different auxin and cytokinin concentrations regenerate an entire new plant [4]. However, recent results suggest that, as in some examples in animals, the regeneration of some plant tissues involves special populations of resident pluripotent cells of restricted potential [5]. Alternatively, plant regeneration might arise through dedifferentiation, such as in callus formation [5]. We highlight below the similarities between different wound-induced cell reprogramming in plants and discuss about the key role of the phytohormone auxin in orchestrating diverse plant regeneration processes.

Abbreviations: ABCB, ATP binding cassette type B; ALF4, ABERRANT LATERAL ROOT FORMATION4; AP2/ERF, APETALA2/ETHYLENE RESPONSIVE FACTOR; ARF, AUXIN RESPONSIVE FACTOR; GH3, GRETCHEN HAGEN3; IAA, indole-3-acetic acid; LBD, LATERAL ORGAN BOUNDARIES DOMAIN; NAC, NAM ATAF1/2 CUC2; PIN, PIN-FORMED; PLT, PLETHORA; PXY, PHLOEM INTERCALATED WITH XYLEM; QC, quiescent center; SCR, SCARECROW; SHR, SHORT ROOT; WIND, WOUND INDUCED DIFFERENTIATION; WOX, WUSCHEL RELATED HOMEOBOX; WUS, WUSCHEL.

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2. Hormonal regulation of transcription factor-asymmetries during wound healing

Plants experience many types of tissue damage in nature, including that caused by herbivory as well as by physical wounding by wind, snow or trampling by animals. Plant cells are surrounded by a rigid wall that fixes their position within tissues, hence they must restore the damaged tissue through cellular regeneration directly at the wound [6]. After tissue wounding, plants activate a plethora of physiological responses required for healing the wounded tissues and protecting them from pathogen infection, *e.g.* induction of pathogenesis-related proteins and wound-related hormones such as ethylene and jasmonate [7]. Unlike other organs, such as leaves or roots, damaged stems must be repaired quickly to restore the physiological functionality of their vascular connections.

2.1. Regulation of tissue repair in partially sectioned plant tissues

Histological time-course studies, differential gene expression and functional analyses helped to unravel some of the physiological and molecular events during wound healing in partially sectioned Arabidopsis stems [8]. In this reference species, wounding interrupted the vascular connection within the stem, and cell divisions were locally induced to rebuild damaged tissues. Auxin was identified as the key factor for wound healing, as tissue reunion was strongly inhibited by global auxin depletion (through decapitation or by inhibition of polar auxin transport, either genetically or pharmacologically) while the exogenous application of auxin to decapitated stems rescued the tissue reunion process [8].

Shortly after differential accumulation of auxin in the upper side of partially sectioned inflorescence stems, *ANAC071*, a NAM, ATAF1/2, CUC2 (NAC)-type transcription factor-encoding gene, was expressed in pith and vascular tissue [8]. A dominant repressor mutant of *ANAC071* had incomplete tissue reunion due to the lack of pith cell division. Consistently with a role of *ANAC071* in activating auxin-mediated pith cell division, the *auxin responsive factor6* (*arf6*) *arf8* double mutants had less cell proliferation in the pith and reduced *ANAC071* levels in incised stems [9]. Additional effectors might be identified through detailed spatial and temporal gene expression analyses using the inducible dominant repressor mutants of *ANAC071*, which are already available [8]. In contrast, *RELATED TO APETALA2 6L* (*RAP2.6L*), encoding a member of the APETALA2 (AP2)/ETHYLENE RESPONSIVE FACTOR (ERF) transcription factor family, was transiently expressed in the lower side of partially sectioned inflorescence stems, and was correlated with a drop in auxin levels [8]. Genetic inactivation of *RAP2.6L* resulted in a moderate inhibition of pith cell proliferation, which disrupted the tissue reunion process.

These results suggest that contrasting auxin levels between adjacent tissues locally activate distinct transcription factors that specify the tissue asymmetries needed for tissue reunion through the local activation of cell proliferation. Additional data indicated that the wound-induced hormones ethylene and jasmonate also contribute to the establishment of these transcription factor-asymmetries. The expression of some genes involved in ethylene and jasmonate production was differentially up-regulated in the upper side or the lower side of partially sectioned inflorescence stems, respectively, where they regulate the expression of *ANAC071* or *RAP2.6L* [8] (Table 1 and Fig. 1A).

Although auxin might directly regulate pith cell proliferation, additional factors are required for complete tissue reunion, as cortical cell divisions were still activated in the absence of either auxin or *ANAC071* function [8]. Experiments in wounded cucumber and tomato hypocotyls suggested that cotyledon-derived gibberellins were required for cell divisions in the cortex during tissue reunion in this organ [10]. Arabidopsis gibberellin-deficient stems showed normal tissue reunion [8]. Thus, the differential hormonal requirement for wound-healing between injured Arabidopsis stems and cucumber or tomato hypocotyls might be due to differences in their developmental context [7] or due to species-specific wound-healing response pathways.

In the current model for tissue reunion of partially sectioned stems (Fig. 1A), wounding creates a discontinuity within the stem that physically interrupts polar auxin transport which in turn produces differential accumulation of auxin in the upper and lower sides of the incision. Accordingly to the canalization hypothesis, where auxin-flow influences the directionality of its own transport through the regulation of rearrangement of auxin polar transporters [11], differential accumulation of auxin in partially sectioned stems might lead to rapid subcellular relocalization of auxin transport components. In experiments performed with wounded pea epicotyls, PIN-FORMED1 (PIN1), the main auxin efflux transporter, rapidly lost its basal polarity in the upper side of the wound but remained basally localized in the lower side, contributing to a quick auxin depletion in this region [12]. In this system, the regeneration of new vasculature strands was preceded temporally and spatially by changes in PIN1 localization, suggesting that auxin levels directly regulate the formation of new vascular tissues after wounding [12]. During normal stem development, new vascular initials are derived from highly orchestrated cell divisions of meristematic tissues (the vascular cambium) located within the vascular bundles [13]. This process is positively regulated by local signaling of the PHLOEM INTERCALATED WITH XYLEM (PXY) leucine-rich repeat receptor-like kinase pathway [14]. The WUSCHEL (WUS)-RELATED HOMEOBOX (WOX) family transcription factors WOX4 and WOX14 are downstream effectors of PXY signaling and control the rate of vascular cell divisions [15]. In addition, WOX4 was proposed to mediate auxin responsiveness of cambial cells for auxin-dependent stimulation of cambial cell divisions [16]. Ethylene and jasmonate have recently emerged as key modulators of cell division in the cambium through their crosstalk with the PXY signaling pathway [17,18]. As far as loss- and gain-of-function mutants are available, these could be used to address whether a similar hormonal crosstalk with the PXY pathway is required for pith cell activation during tissue reunion on partially sectioned stems.

So far, regeneration in partially sectioned Arabidopsis stems seems to be epimorphic, as extensive cell proliferation anticipates tissue reunion. An issue that has not yet been fully addressed is whether these proliferating cells arise by cell dedifferentiation or by activation of resident meristematic cells (*i.e.* cambium), or both. Whole-organ lineage tracing in wild-type and selected mutant plants, combined with analyzing the spatial and temporal patterns of cell divisions at the injured stems, as it has been recently performed in lateral root initiation using light sheet-based fluorescence microscopy [19], should be implemented for a full understanding of the cellular and molecular events occurring during tissue reunion at a cellular resolution.

2.2. Cell-to-cell communication for graft formation and vascular reconnection

Grafting is an ancient plant propagation technique mostly accomplished by connecting two different plant tissues, the shoot (scion) and the root (rootstock), from the same or different species with the aim of improving crop management and productivity [20]. In addition, grafting has long been applied for long-distance signaling research in many plant processes, including regulation of flowering time, shoot branching and virus-induced gene silencing [21]. Whereas intraspecific grafts (homografts) are presumably always compatible, interspecific grafts (heterografts) usually lead