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Shoot regeneration: a journey from acquisition of competence to completion

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Plants display an extraordinary ability to regenerate complete shoot systems from a tissue fragment or even from a single cell. Upregulation of the determinants of pluripotency during a precise window of time in response to external inductive cues is a key decisive factor for shoot regeneration. A burst of recent studies has begun to provide an understanding of signaling molecules that are instrumental in the making of the regenerative mass, as well as the developmental regulators that are seminal in shaping the pluripotent state. Here, we discuss how signaling molecules, waves of mutually exclusive stem cell regulators and epigenetic modifiers could contribute to cellular heterogeneity in an island of regenerative mass, thus leading to *de novo* shoot regeneration.

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Introduction

A zygote exemplifies a single cell capable of giving rise to a complete organism. However, plants are among the rare organisms known to possess the extraordinary capacity of awakening the totipotency that lies dormant in a few selected cells of the adult body [1•,2,3]. The current scale of plant regeneration studies, and commercial applications resulting from them, arises from our understanding of the crucial role played by two key plant hormones, auxin and cytokinin, in modulating *de novo* plant regeneration [4]. *De novo* organogenesis [5,6] and somatic embryogenesis [7] are strictly hormone-induced and hormone-controlled processes that are almost impossible without these external cues. External inductive cues of this nature, which are sufficient for cellular reprogramming to give rise to complete organism from a tissue fragment, are limited to the plant kingdom (Figure 1) [3,8]. Over the years, several studies of the loss of function of key plant specific transcription factors and the consequences of their forced expression during *de novo* shoot regeneration have permitted the unearthing of the underlying molecular mechanisms [1•,9,10••,11,12•,13,14]. In this review, we highlight how recent studies into *de novo* shoot regeneration have begun to reveal the fundamental cellular and molecular events in real time, and how this has generated renewed interest in novel areas of reprogramming [15], where a number of key questions yet remain unsolved.

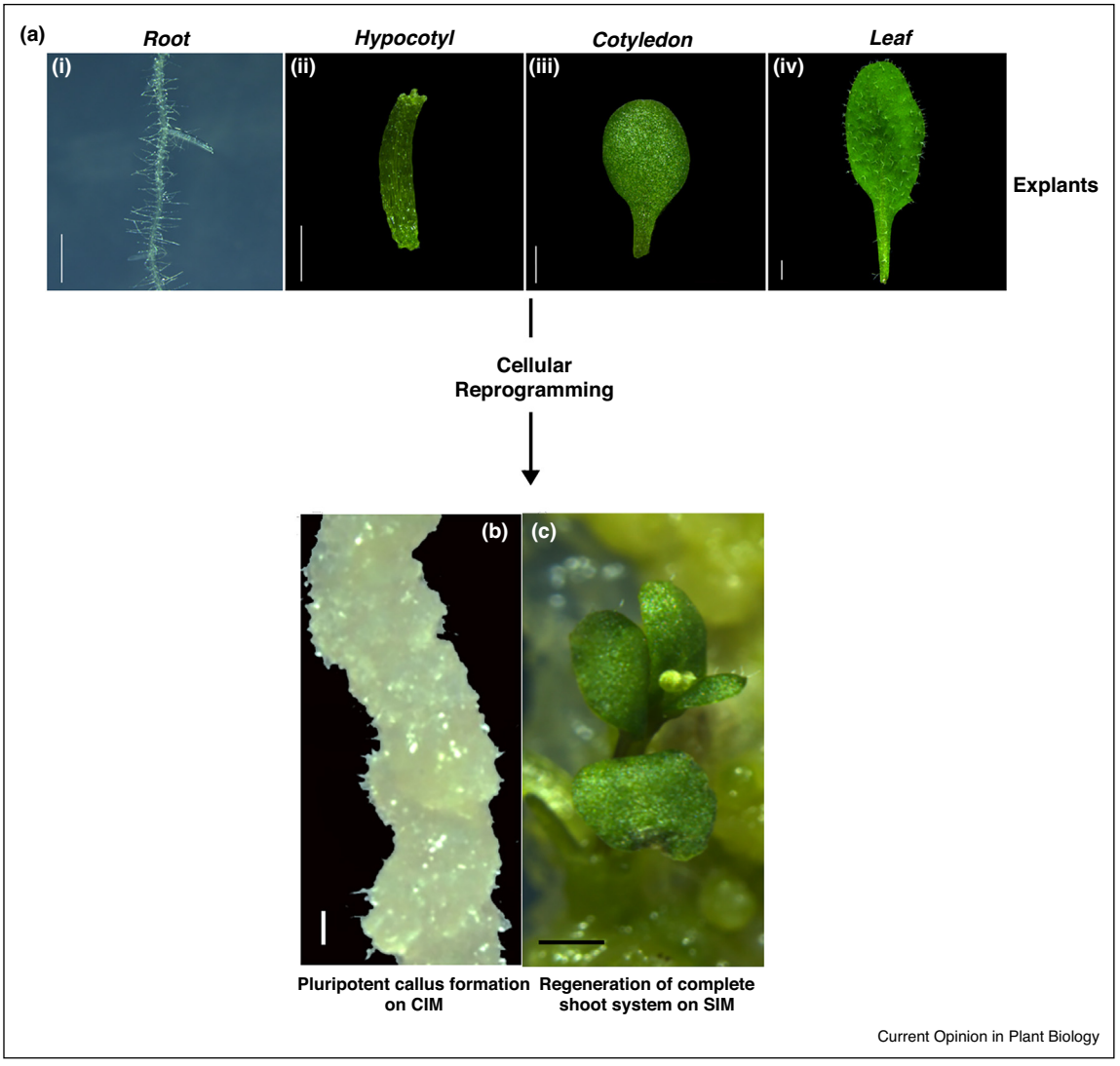
Modes of regeneration

Until now, the most investigated and best understood mode of shoot regeneration is callus mediated regeneration using a two step protocol or a modified version of this protocol [5]. Indirect *de novo* shoot regeneration involves two phases of incubation: first callus induction on an auxin rich medium, followed by shoot organogenesis on a cytokinin rich medium [10••,16–19]. The regenerative potential of explants used for callus induction largely depends on their developmental stages [18,20] and origins [5,21]. In contrast, direct shoot organogenesis involves the incubation of suitable explants on cytokinin containing media, with or without prior auxin priming [10••,16,22,23••,24]. Conversion of lateral root primordia (LRP) to shoots is an example of direct regeneration. The central role of each hormone, in moulding the plasticity of plant cells, is demonstrated by the critical importance of either auxin or cytokinin during the different phases of incubation during both modes of regeneration.

Acquisition of competence to regenerate shoot in response to relative abundance of plant hormones

The auxin to cytokinin ratio in the culture medium is key to the development of a pluripotent callus [16,25••]. Several lines of evidence demonstrate an irreplaceable role for high auxin concentration in LRP initiation [26] as well as in pluripotent callus formation [27]. The organogenetic potential and the molecular characteristics of a callus induced on a cytokinin rich callus induction medium (CIM) is markedly different from a callus induced on auxin rich CIM. High concentrations of cytokinin induces phloem pericycle cell divisions in

Figure 1



Arabidopsis explants undergoing cellular reprogramming to regenerate complete shoot systems. **(a)** Explants used for *de novo* shoot regeneration: (i) root, (ii) hypocotyl, (iii) cotyledon, and (iv) leaf. **(b)** Pluripotent callus formation from explant on callus induction medium (CIM). **(c)** Regeneration of complete shoot system from callus on shoot induction medium (SIM). Scale bars in (a.ii and a.iii) represent 0.5 mm and 1 mm in the rest of the images. Image (c) is reprinted from Kareem *et al*. [10••] with permission from Cell Press. License number: 4158680029029.

contrast to the xylem pole pericycle divisions induced on auxin rich media [16]. Key root trait determinants, essential for the establishment of pluripotency (see the section below), are dramatically downregulated in cytokinin rich CIM and such callus fails to regenerate shoots [16,25••]. These studies suggest the sole use of cytokinin in CIM is not sufficient to drive the downstream molecular changes and cell divisions required to establish the pluripotency and thus the ability to regenerate shoots. During direct regeneration, auxin rich LRP are competent to make shoots when exposed to high cytokinins [10••,16,23••,24], further supporting the role of high auxin in establishing the pluripotency necessary to regenerate shoots.

A root developmental pathway is important for callus formation

Initial incubation on CIM prompts the pool of reprogrammable adult stem cells constituted by xylem-pole pericycle cells in roots, and pericycle-like cells in aerial explants, to undergo rapid division in response to exogenously applied auxin [25••,28,29]. These cell divisions and ensuing molecular changes during CIM pre-incubation play a critical role in the acquisition of competence for shoot induction [10••,16,17,19,25••,29]. As these cells proliferate, the expression of *J0121* [30], a marker for differentiated pericycle cells, becomes diffuse and disappears from the callus [17,25••,29]. The disappearance