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Article Addendum

Reprogramming adult cells during organ regeneration in forest species

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The possibility of regenerating whole plants from somatic differentiated cells emphasizes the plasticity of plant development. Cell-type respecification during regeneration can be induced in adult tissues as a consequence of injuries, changes in external or internal stimuli or changes in positional information. However, in many plant species, switching the developmental program of adult cells prior to organ regeneration is difficult, especially in forest species. Besides its impact on forest productivity, basic information on the flexibility of cell differentiation is necessary for a comprehensive understanding of the epigenetic control of cell differentiation and plant development. Studies of reprogramming adult cells in terms of regulative expression changes of selected genes will be of great interest to unveil basic mechanisms regulating cellular plasticity.

In plants, the possibility to regenerate roots, shoots or embryos directly from cells other than root or shoot meristems, lateral root initials or zygotes has been known for more than 60 years and has been exploited in horticulture, agriculture and forestry.[1] However, little is known about the mechanisms that enable a somatic differentiated cell to switch its fate into a multipotent, pluripotent or totipotent cell that can develop a root, shoot or embryo or repair damaged tissues. Although apparent dedifferentiation and respecification of cells seems to occur,[2] whether acquisition of competence to regenerate organs occurs, as in animal cells,[3] through dedifferentiation,[4] via transdifferentiation[5] or by the use of pre-existent totipotent, pluripotent or multipotent cells[6] in adult tissues remains unknown. Sena et al.[7] have described in *Arabidopsis thaliana* that a reversion to stem cell niche activity is not necessary for in vivo early-root tip regeneration from developmentally young cells that have undergone only few divisions after the asymmetric division of a stem cell, and a set of markers associated with the competence of tissues is reported. However, Zhao et al.[8] describe the formation of a stem cell niche from competent callus cells obtained from young seedlings during in vitro plant organogenesis. According to Birnbaum and Sánchez-Alvarado,[2] there could be many possible pathways and strategies from early to later stages of regeneration in both animals and plants depending on the progenitor cells. However, despite the diverse regeneration mechanisms, almost all phenomena in one kingdom seem to have a counterpart in the other. Therefore, another interesting point would be to understand the mechanisms of respecification of fully differentiated progenitor cells that do not pass through a callus state to regenerate (direct organogenesis), especially in relation to the developmental age of cells and tissues. It is well known that regeneration efficiency is much higher in tissues at earlier stages of development.[9,10] Even callus cells from adult tissues of many species maintain the committed state of the progenitor cells showing a lower proliferation rate and lower regeneration capacity than those induced from juvenile tissues.[11] This raises the question of whether plants maintain certain cells, which have not been determined to develop a specific organ, outside the meristematic region in a specific differentiated state that can easily access pluripotent or multipotent properties, and whether callus cells loose the differentiated characteristics of the tissue from which they arose.

In forest species, a decline in the capacity of regenerating shoots, roots or embryos from somatic differentiated cells is stably associated with tree age and maturation.[12-14] The loss of regeneration capacity associated with the juvenile-adult transition makes forest species representative and reliable systems to study how cell fate becomes fixed during development and how plant cells can manage to retain developmental plasticity.

The decline in the capacity to regenerate roots from cuttings is one of the most dramatic effects of tree maturation and has been the subject of investigations on the basic nature of the process.[15] Adventitious root formation is a postembryonic organogenic process in which roots are induced, in general, from determined or differentiated cells that have not been specified to develop a root at positions where they do not normally occur during development. Adventitious root formation is usually induced in stem cuttings, which experience a stimulus, such as wounding, although it can also take place in intact plants in some species. Using a very simple experimental system based on the different

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rooting capacity of hypocotyl and epicotyl cuttings from young seedlings of pine, a few clues about the mechanisms underlying the process have been obtained.[13] The system takes advantage of the different regeneration capacity of similar cell types at different developmental stages, and the abscence of callus formation during the regeneration process. Therefore, a direct developmental switch, without passing through a developmentally non-identified callus cell, can be studied.

In our system, cells competent to form adventitious roots are confined to cambial region of the hypocotyl located centrifugal to the resin canal at the xylem poles. These cells exhibit rapid cell division and re-orientation of division planes to organize a root meristem in response to exogenous auxin. The early cellular responses to auxin are similar in rooting competent and non-competent cells, which show cell reorganization and mitotic activity that spreads out into the cortex. However, rapid cell division and reorientation of division planes preceeding root meristem organization are observed in a short period of time (6d) only in competent cells at the mentioned positions. Therefore, auxin-induced root meristem organization appears to occur independently of cell reorganization and division.[16] Cell fate switches are commonly regarded as being associated with the re-entry of cells into the cell cycle.[17] However, from the data obtained it seems that the capacity to re-enter cell division alone is not sufficient to reset the previous cellular state in non-competent cells. Because rooting competent and non-competent cells respond similarly during the earliest stages of root induction, it was concluded that cambial cells peripheral to the resin canals might retain an intrinsic competence to form adventitious roots in response to auxin, and perhaps maintain or regain their pluripotency/multipotency before cell division or during the initial cycles of division.[13,16]

Further insights into the control of adventitious root meristem formation in pine have been obtained by studying the pattern of induction of potential regulatory genes. Cell fate switches both in plants and animals are characterized by remarkable changes in the pattern of gene expression, as cells switch from an expression pattern typical of a somatic cell to a new one directing a new developmental pathway.[18,19] Thus, determining the way by which cells reset their gene expression pattern, especially for the timetable and repertoire of gene expression characteristic of the earliest stages of normal development, is crucial to understand cellular plasticity.[7,20,21] Therefore, in the case of rooting, regulatory genes underlying root meristematic pluripotent stem cell initiation and maintenance during development might be candidate genes involved in the step of determination towards root meristem from progenitor adult cells. A group of such genes encode GRAS proteins, transcription factors involved not only in root patterning but also in the establishment of the quiescent centre identity and in the maintenance of the stem cell status of the surrounding initial cells during the embryonic pattern formation and postembryonary development.[22,23] An experimental design based on the analysis of temporal and spatial expression of two pine genes encoding GRAS proteins in rooting competent and non-competent cuttings of pine provided additional clues to the process.[24,25] Low but detectable levels of mRNA were detected for both genes in competent and non-competent tissues indicating that there is no parallelism between the levels of mRNA and the decrease in rooting potential. However, a transient increase of mRNA levels asymmetrically localized to the cambial area and rooting competent cells was only observed in rooting competent cuttings during the earliest stages of adventitious root induction, before the resumption of cell division leading to the root formation pathway. In addition, there was an overlap in the temporal and spatial expression domain of both genes in competent cuttings indicating possible functions in the same tissues at the same time, in agreement with the cascade of *GRAS* genes involved in root meristem specification and maintenance that has been described in model plant species.[26] Furthermore, differential responses to exogenous auxin suggest that both auxin-dependent and independent pathways could be involved in the root induction process. However, mRNA levels asymmetrically increased in the cambial region and rooting competent cells were not detected in non-competent cuttings. In these cuttings, expression spread out into the cortex and dividing cells.

Based on these results, it seems that pathways involving GRAS proteins, which can interact or work independently, can be induced in tissues involved in rooting during the earliest stages of adventitious root formation. In addition, the difference in competence to increase mRNA levels asymmetrically during the earliest stages of adventitious root formation in similar cell types at different developmental stages suggests the presence of specific cellular signalling pathways or specific factors, perhaps distributed in a localized- or developmental-specific manner, in the tissues involved in rooting that could be crucial for rooting capacity. The nature of these signalling pathways or factors is unknown. Since auxin transport, accumulation and metabolism do not account for the difference in the rooting capacity of pine cuttings,[13] other factors such as the chromatin status and epigenetic mechanisms resulting in a specific nuclear architecture could be involved in the control of age-dependent cellular plasticity.[27,28] Chromatin remodelling factors possibly required for the removal of epigenetic markings could be crucial for genome resetting and reprogramming during cell fate switch resulting in the increased mRNA levels of tissue-specific genes in a position-dependent manner.[29]

Changes in cellular plasticity in plants may occur both with and without cell division, in the same way as in animals.[17] Localized changes of *GRAS* gene expression in competent cells during the earliest stages of adventitious root formation, when cell reorganization occurs but before the resumption of cell division, indicates differential cellular responses between competent and non-competent tissues before cellular division. This suggests that these cells have the capacity to reset their expression pattern, at least for specific genes, and, perhaps, to make developmental decisions, before cell division. It is not clear whether this response represents a nuclear reprogramming toward the formation of a root cell or a dedifferentiation process, either of them required for re-entering a formative cellular division pathway in competent tissues, as opposed to the proliferative cellular division pathway in non-competent tissues.[30] Perhaps non-competent tissues require more rounds of cell division or changes in the duration of the cell cycle progression phases to reset epigenetic marks or other determination

factors leading to switches in cell fate, as described in Drosophila.[31] In this case, numerous cycles of cell division could be necessary to reset the previous cellular commitment.

Unveiling the early events leading to the acquisition of cell pluripotency/multipotency is a fundamental issue to understand cellular plasticity. Searching for master regulatory genes is essential in the future of adult cell fate switch studies. These genes would control the step of determination towards organ formation of progenitor adult cells. But equally important is to search for master rechanneling genes which could be acting at the earliest events of fate switching. Their function could be related to the cancellation of pre-existing expression pathways, which would be followed by the activation of master regulatory genes for the expression of new specifities channeling the direction of destabilized differentiated cells. Genes such as *Oct4*, *Sox2* or *Nanog* that encode transcription factors specifying embryonic stem cell identity in animals, could fall in this category.[32] Overexpression of some of these genes has been used to reprogramme adult somatic cells to pluripotent embryonic stem-like cells.[29] Understanding the mechanisms regulating how plant cells can switch fate and the possibility to compare the mechanisms regulating cellular plasticity between plants and animals could help to find new strategies to improve animal and plant regenerative capacity, which could have a great impact on medicine, agriculture and forestry.

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References

1. Vasil IK. A history of Plant Biotechnology: from the cell theory of Schleiden and Schwann to biotech crops. Plant Cell Rep 2008; 27:1423-40.
2. Birnbaum KD, Sanchez Alvarado A. Slicing across kingdoms: regeneration in plants and animals. Cell 2008; 132:697-710.
3. Stocum DL, Zupane GKH. Stretching the limits: Stem cells in regeneration science. Dev Dyn 2008; 237:3648-71.
4. Rideout WMR, Eggan K, Jaenisch R. Nuclear cloning and epigenetic reprogramming of the genome. Science 2001; 293:1093-8.
5. Thowfeequ S, Myatt EJ, Tosh D. Transdifferentiation in developmental biology, disease, and in therapy. Dev Dyn 2007; 236:3208-17.
6. Yiang Y, Jahagirdar BN, Reinhardt L, Schwartz RE, Keene CD, Ortiz-Gonzalez XR, et al. Pluripotency of mesenchymal stem cells derived from adult marrow. Nature 2002; 418:41-9.
7. Sena G, Wang X, Liu HY, Hofhuis H, Birnbaum KD. Organ regeneration does not require a functional stem cell niche in plants. Nature 2009; 457:1150-4.
8. Zhao XY, Su YH, Cheng ZJ, Zhang XS. Cell fate switch during in vitro plant organogenesis. J Integr Plant Biol 2008; 50:816-24.
9. Díaz-Sala C, Rey M, Rodríguez R. Establishment of a cycloclonal chain from nodal segments and apical buds of adult hazel. Plant Cell Tiss Organ Cult 1990; 23:151-7.
10. Guzzo F, Baldan B, Mariani P, LoSchiavo F, Terzi M. Studies on the origin of totipotent cells in explants of *Daucus carota* L. J Exp Bot 1994; 45:1427-32.
11. George EF, Hall MA, De Klerk GJ. Plant Propagation by Tissue Culture. Volume 1. 2008; The Background. 3rd Edition. Exegetics, Basingstoke UK.
12. Greenwood MS, Hutchison KW. Maturation as a developmental process. *In* Clonal Forestry: Genetics, Biotechnology and Application. Ahuja MR, Libby WJ, (eds), Springer Verlag, New York 1993; 14-33.
13. Díaz-Sala C, Hutchison KW, Golfbarb B, Greenwood MS. Maturation-related loss in rooting competence by loblolly pine stem cuttings: role of polar auxin transport and tissue sensitivity. Physiol Plant 1996; 97:481-90.
14. Day M, Greenwood MS, Díaz-Sala C. Age-and size-related trends in woody plant shoot development: regulatory pathways. Tree Physiol 2002; 22:507-13.
15. Hutchison KW, Singer PB, McInnis S, Díaz-Sala C, Greenwood MS. Expansins are conserved in conifers and expressed in hypocotyls in response to exogenous auxin. Plant Physiol 1999; 120:827-31.
16. Greenwood MS, Cui X, Xu F. Response to auxin changes during maturation-related loss of adventitious rooting competence in loblolly pine (*Pinus taeda*) stem cuttings. Physiol Plant 2001; 111:373-80.
17. Costa S, Shaw P. "Open minded" cells: how cells can change fate. Trends Cell Biol 2006; 17:101-6.
18. Thibaud-Nissen F, Shealy RT, Khanna A, Vodkin LO. Clustering of microarray data reveals transcript patterns associated with somatic embryogenesis in soybean. Plant Physiol 2003; 132:118-36.
19. Terranova R, Pereira CF, Du Roure C, Merkenschlager M, Fisher AG. Acqusition and extinction of gene expression programs are separable events in heterokaryon reprogramming. J Cell Sci 2006; 119:2065-72.
20. Corti S, Locatelli F, Papadimitriou D, Strazzer S, Bonato S, Comi GP. Nuclear reprogramming and adult stem cell potential. Histol Histopathol 2005; 20:977-86.
21. Che P, Lall S, Nettleton D, Howell SH. Gene expression programs during shoot, root, and callus development in arabidopsis tissue culture. Plant Physiol 2006; 141:620-37.
22. Helariutta Y, Fukaki H, Wysocka-Diller J, Nakajima K, Jung J, Sena G, et al. The *SHORT-ROOT* gene controls radial patterning of the Arabidopsis root through radial signalling. Cell 2000; 101:555-67.
23. Sabatini S, Heidstra R, Wildwater M, Scheres B. SCARECROW is involved in positioning the stem cell niche in the Arabidopsis root meristem. Genes Dev 2003; 17:354-8.
24. Sánchez C, Vielba JM, Ferro E, Covelo G, Solé A, Abarca D, et al. Two *SCARECROW-LIKE* genes are induced in response to exogenous auxin in rooting-competent cuttings of distantly related forest species. Tree Physiol 2007; 27:1459-70.
25. Solé A, Sánchez C, Vielba JM, Valladares S, Abarca D, Díaz-Sala C. Characterization and expression of a *Pinus radiata* putative ortholog to the Arabidopsis *SHORT-ROOT* gene. Tree Physiol 2008; 28:1629-39.
26. Xu J, Hofhuis H, Heidstra R, Sauer M, Friml J, Scheres B. A molecular framework for plant regeneration. Science 2006; 311:386-8.
27. Grafi G. How cells dedifferentiate: a lesson from plants. Dev Biol 2004; 268:1-6.
28. Verdeil JL, Alemanno L, Niemenak N, Tranbarger TJ. Pluripotent versus totipotent plant stem cells: dependence versus autonomy?. Trends Plant Sci 2007; 12:245-52.
29. Jaenisch R, Young R. Stem cells, the molecular circuitry of pluripotency and nuclear reprogramming. Cell 2008; 132:567-82.
30. Veit B. Stem cell signalling networks in plants. Plant Mol Biol 2006; 60:793-810.
31. Sustar A, Schubiger G. A transient cell cycle shift in Drosophila imaginal disc cells precedes multipotency. Cell 2005; 120:383-93.
32. Chi AS, Bernstein BE. Pluripotent chromatin state. Science 2009; 323:220-1.