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Addendum

Factors that Mediate Root Initiation in Plants

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Addendum to:

Factors involved in root formation in Medicago truncatula

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ABSTRACT

When the leaf explants of *Medicago truncatula* are placed on auxin medium they form root initials within a week. Our studies have shown that the cells associated with the veins are stimulated into division by the added auxin, forming what we called vein-derived cells (VDCs) that grow out into callus and it is from these cells that the root meristems are formed. The way auxin interacts with other hormones is a key factor in determining the stem cell fate. In *Medicago truncatula* if cytokinin is added with auxin then root production is blocked and embryos are produced along with many more vascular tissues are produced throughout the callus. However, if the explants are exposed to auxin for seven days before the addition of cytokinin then only roots will form and not embryos. Thus, the commitment stage once induced is irreversible. Our study suggested that a pool of procambial or stem cells exist in the vascular tissues of the leaf and that these can be stimulated into pluripotency by auxin addition and the number of roots formed from the same leaf explants could be enhanced by added oxidized or reduced glutathione or an alteration of the ethylene sensitivity.

Roots serve a multitude of functions such as anchorage, as the conduit to supply both nutrients and water to the plant from the soil, as a location for the synthesis and exchange of various plant hormones, as storage organs of plant resources.[1] Plant roots grow in a highly heterogeneous environment—the soil, and possess an ability to react to this heterogeneity and modify the form of their root system as a consequence. This is a "phenotypic plasticity" which is influenced by a genetic program and environmental factors and the ultimate configuration of the root system. To understand the morphogenesis of roots it is necessary to define the organization of the root meristem and attempt to determine the fate map of cells emerging from the root meristem.[2] As the majority of the root system consists of lateral roots, which is controlled by various, genetic, hormonal and nutritional factors. Primary root growth occurs from the root apical meristem (RAM) and is dependent on a stem cell niche or microenvironment being established giving rise to the quiescent centre (QC).[3] The RAMs are maintained by retaining a stem cell reservoir and a pool of undifferentiated initial cells.

When the leaf explants of *Medicago truncatula* are placed on auxin medium they form root initials/primordia within a week. Our studies have shown that the cells associated with the veins are stimulated into division by the added auxin, forming what we called vein-derived cells (VDCs) that grow out into callus and it is from these cells that the root meristems are formed.[4] Differentiation proceeds from the meristems to produce the distinctive tissues of the root. These VDCs appear to be derived from the cells of or near the phloem. The way auxin interacts with other hormones is a key factor in determining the stem cell fate. In *M. truncatula*, if cytokinin is added with auxin then root production is blocked and embryos are produced along with many more vascular tissues are produced throughout the callus. However, if the explants are exposed to auxin for seven days before the addition of cytokinin then only roots will form and not embryos. Thus, the commitment stage once induced is irreversible. Our study suggested that a pool of procambial or stem cells exist in the vascular tissues of the leaf and that these can be stimulated into pluripotency by auxin addition. Our other studies showed that the number of roots formed from the same leaf explants could be enhanced by added oxidized or reduced glutathione or an alteration of the ethylene sensitivity (Fig. 1). How can these bioactive agents do this? Does it mean that there are stem cells that have different thresholds of requirements to commit them to differentiate? An important question is whether these stem cell precursors are actually a group of cells held in a developmental check by the adjacent leaf cells? That is, is this a form of "positional information" control of differentiation within the leaf as

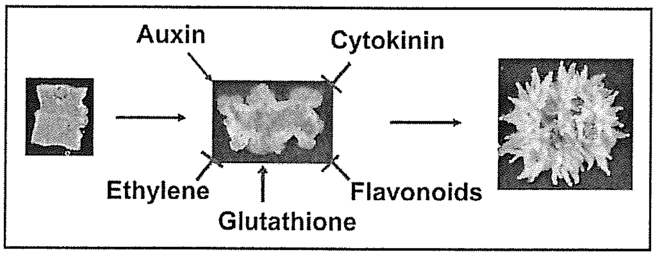

Figure 1. In vitro root initiation in model legume Medicago truncatula. Hormones such as auxin, cyokinin and ethylene and other bioactive compounds such as flavonoids and glutathione are essential in regulating root primordia formation.

occurs in animal embryology where cells respond as though they appreciate their spatial positions in the embryo? Perhaps in the leaf vascular bundle, which consists of parenchyma sheath cells, xylem and phloem cells, their neighbours and position in the bundle hold the proposed procambium cells in check? Hence, the way auxin interacts with other hormones is a key factor in determining the stem cell fate. These observations are reminders of legume nodule formation and lateral root formation. *Rhizobia* induce root cell commitment to an out growth called a nodule. There is a numerical nodule numbers control per root but this can be over come by environmental treatments and the appropriate plant mutants.[5] The question is whether the initial cells committed by *Rhizobium* signals are latent stem cells in the root? Such knowledge would be basic to the adaptation of legumes to the harsh environment, root development for soil remediation, soil moisture and nutrient exploration and ultimately plant yield.

Ethylene is involved in the commitment of leaf explants to root formation in response to auxin. The loss of ethylene sensitivity, in *skl* mutant which is ethylene-insensitive and defective in ethylene signalling, enables a marked increase in successful root formation and little tracheid formation. The enhanced auxin-stimulated root formation in the *skl* mutant suggests that blocking ethylene transduction in this mutant enhances root meristem initiation by enabling more stem cells to differentiate in this direction; rather than producing vascular (tracheid) tissue.[4,6] The simplest explanation is that ethylene (via the *skl* gene product, or processes affected by it) acts as a negative regulator of commitment to the root developmental pathway. Similar mode of action possibly occurs in early nodule development, when the plant induces ethylene-mediated stress responses to limit nodule numbers. We proposed that the ethylene inhibition is most likely to affect the stage of development where the formation of the initial files of early vascular tissues occurs.

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