

# Root branching plasticity: collective decision-making results from local and global signalling

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Cells within tissues can be regarded as autonomous entities that respond to their local environment and to signals from neighbours. Coordination between cells is particularly important in plants, as the architecture of the plant adapts to environmental cues. To explain the architectural plasticity of the root, we propose to view it as a swarm of coupled multicellular structures, rhizomers, rather than a large set of autonomous cells. Each rhizomer contains a primed site with the potential to develop a single lateral root. Rhizomers are spaced through oscillatory genetic events that occur at the basal root tip. The decision whether or not to develop a lateral root primordium results from the interplay between local interactions of the rhizomer with its immediate environment, such as local nutrient availability, long-range interactions between the rhizomers and global cues, such as overall nutrient uptake. It can halt lateral root progression through its developmental stages, resulting in the observed complex root architecture.

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Current Opinion in Cell Biology 2017, 44:51–58

This review comes from a themed issue on **Cell Architecture**

Edited by **Terry Lechler** and **Rafael Carazo Salas**

For a complete overview see the [Issue](#) and the [Editorial](#)

Available online 25th March 2017

<http://dx.doi.org/10.1016/j.ceb.2017.03.001>

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## Introduction

Botanists have long considered the modularity of plants, that is, having a body-plan composed of subunits that retain an essence of the whole [1–3]. The concept is based on a well-defined physiological unit — a phytomer — that is repeated to generate the regular phyllotactic patterning of buds, leaves and branches [4]. Such patterning is in stark contrast to the irregular spacing of lateral roots along the primary root axis, suggesting the phytomer concept is inadequate to describe root development. In this paper we discuss hypotheses to explain root plasticity and where they

fall short. Based on recent molecular findings, we propose an unifying hypothesis for the communication underlying lateral root formation. It views the root as a collection of subunits, which we coin rhizomers, which interact using local and long-distance communication to achieve functional rhizotaxy.

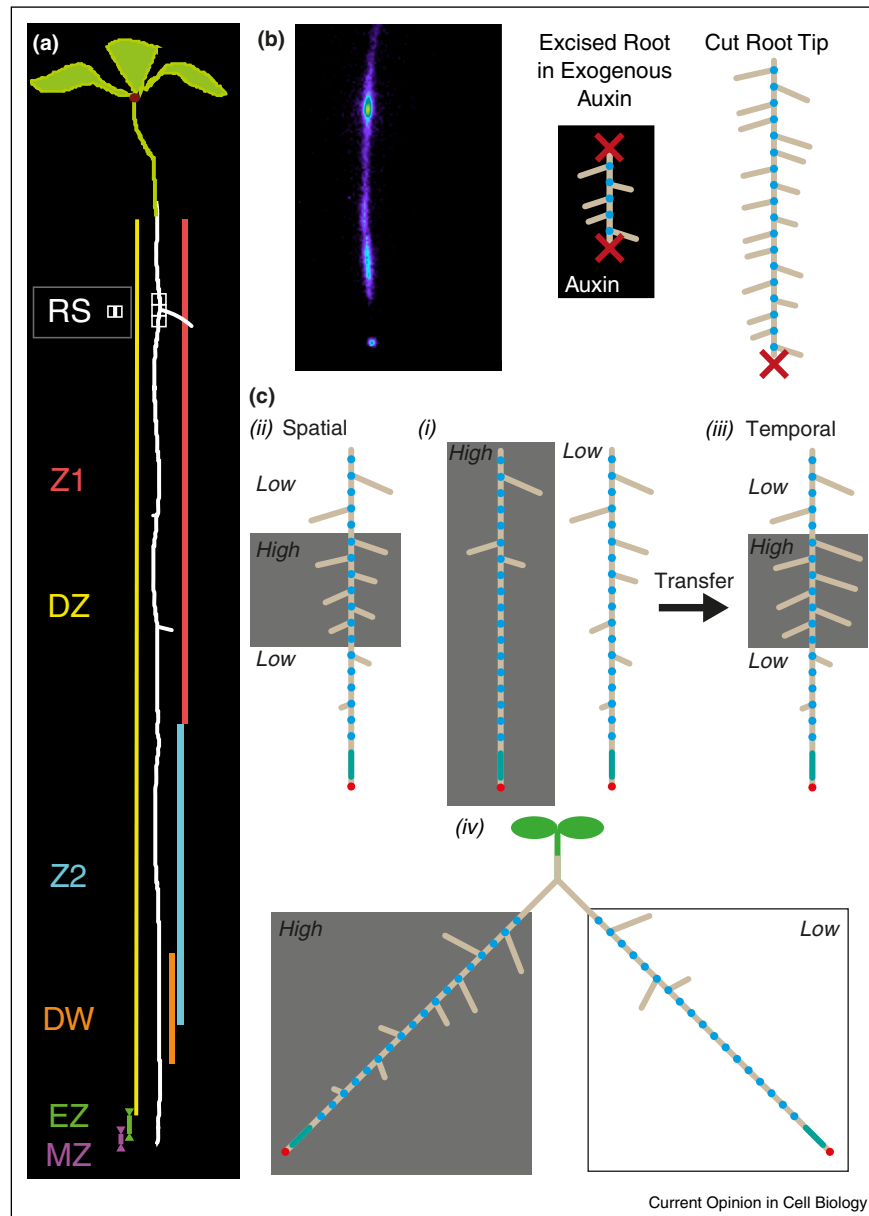
## Acropetal patterning: primed at the right place at the right time

Root architecture is a critical component of a plant's strategy to survive, representing the adaptation to the available nutrients in the soil. In the model plant *Arabidopsis* the root system consists of the shoot-connected primary root and the nutrient-foraging lateral roots, LRs (Figure 1a). LRs progress through, roughly, three physiological processes: priming, initiation, and emergence/elongation. Priming occurs when the growing primary root tip leaves a template of regularly spaced prebranch sites — cells expressing increased response to the phytohormone auxin (Figure 1b). The periodic pattern results from temporally oscillating genes in the oscillation zone (OZ), which is contained within the meristematic zone (MZ) and elongation zone (EZ) of the root (Figure 1a, [5]). A recent study suggests that the observed oscillatory behaviour could result from auxin influx into the vasculature originating from root cap cells undergoing programmed cell death [6]. Only at primed, prebranch sites LR formation can initiate, through an anticlinal cell division of one of the xylem-pole pericycle cells. Dubrovsky *et al.* [7] experimentally inferred a developmental window corresponding to the length along the primary root axis along which a primed site can initiate the formation of a lateral root primordium (LRP). According to their measurements, the developmental window exists for roughly 10.4 hours, positioned on average about 3.95 mm from the root tip's stem cells (Figure 1a). Further analysis revealed that no LRP are initiated *de novo* between two already established LRPs or LRs. Moreno-Risueno *et al.* [8] found that all primed sites also initiated, while subsequent emergence was subject to additional patterning. These data suggest that initiation naturally follows priming and always occurs acropetally within a narrow developmental window.

## Basipetal patterning: breaking the sequence in LR emergence

The regular priming pattern at the root tip (Figure 1b) resembles in many ways the auxin prepatterning

Figure 1



Root plasticity: LR developmental zones and Arabidopsis rhizotaxy. **(a)** Developmental zones in the root of a 7–8 day old seedling are labelled along the primary root axis: meristematic zone (MZ); elongation zone (EZ); differentiation zone (DZ); LR developmental window (DW); LR developmental zone 1 and 2 (Z1 and Z2, respectively). Lengths and positions of MZ, EZ and DZ are approximated from data in [14]; DW, Z1 and Z2 are approximated from data in [7]. Approximate rhizomer size (RS, white box) is calculated from a 7 day-old root growth rate and oscillation period. **(b)** Overlay of DR5::Luciferase and brightfield images of a 5-day-old root shows LRs emerging from DR5-marked prebranch sites. Occasionally a prebranch site fails to develop into an emerged LR (asterisk) (image from [8]). All prebranch sites emerge in cut-tip roots or when induced with exogenous auxin. **(c)** LR development is regulated by available nutrient conditions, in this case different nitrate availabilities. (i) Homogeneous high nitrate levels inhibit LR emergence while homogeneous low nitrate levels promotes LR emergence. (ii) The impact of a temporal change. A root grown under homogeneous low nitrate levels and transferred to heterogeneous nitrate levels increases LR emergence specifically within the high nitrate zone. (iii) The impact of a spatial change. A root that grows through heterogeneous patches of low, high and low nitrate levels presents more LR emergence in the high nitrate zone. (iv) A split-root experiment with a single plant simultaneously growing primary root axes under both high and low nitrate conditions, resulting in more LR emergence in the high nitrate region compared to the low nitrate region. Blue dots indicate LRP; red dots, MZ; green bars, EZ; red 'X', cut root.

underlying phyllotaxis at the shoot apical meristem. This analogy, however, breaks down during the subsequent development of LRP. LRP must progress through eight developmental stages until becoming fully emerged [9]. Uninterrupted, LRP development can take about 40 hours [10]; this developmental journey is however not guaranteed, nor is it necessarily continuous. Dubrovsky *et al.* [7] found that in 8-day-old plants within the region of emerged LRs ('zone 1' in Figure 1a) only 66% of the LRP had emerged, with an additional 11% emerging seven days later. Thus, despite acropetal initiation, LRP may develop and emerge basipetally, between previously emerged LRs. This raises a conundrum: if LR initiation occurs strictly acropetally, then how is development of LRP at least partly modulated basipetally? Essentially, to what extent is root architecture dictated by the patterning dynamics at the root tip and to what extent by systemic control of the shoot, or possibly other factors? Further insights are offered by experiments in which basipetal development is promoted.

## The plasticity puzzle

### The importance of auxin

Basipetal development does not seem to be a mere adjustment of an otherwise linear rate of LRP development. Root extract experiments — in which a small piece of the primary root axis is excised and incubated in exogenous auxin (Figure 1b) — demonstrate emergence of LRP in response to auxin, despite isolation from the remainder of the plant [11]. Likewise, removal of the root tip causes rapid auxin accumulation, inducing the emergence of all LRP (Figure 1b). These experiments corroborate the idea [5] that all prebranch sites initiate within the developmental window, but may remain dormant until emergence is induced by auxin accumulation. In fact, other phytohormones such as cytokinin are known to regulate LRP development as well [11]. We begin by evaluating current hypotheses to account for root plasticity through sequential initiation and strategic dormancy of LRP.

### Knowing when to grow: a LRP must communicate

Multiple hypotheses have emerged to explain LR plasticity [3,5,12], yet none of them can account for all standard rhizotactic patterns under different growth conditions. There might be a combination of mechanisms at work. For example, Scheres and Laskowski [13] proposed that the 'bending hypothesis' (see below) and 'oscillation hypothesis' do not have to be mutually exclusive. We extend such reasoning by looking at the requirements for a general hypothesis for root plasticity to account for rhizotaxy under different non-standard growth conditions. Although LRs typically develop acropetally, the root's plastic capacity is readily unveiled under more challenging growth conditions that better resemble growth in a natural soil environment. First, mechanical bending studies show that LRs can be induced at the bends. It has been

established that LRP are intrinsically promoted to develop at curves, through either mechanotransduction or geometrically induced phytohormone redistribution [14,15]. Although opinions diverge as to what unleashes the curvature-induced responses, the observation itself aligns with the idea of prepatterned periodic prebranch sites induced to emerge through signalling. Bending studies strongly support the notion that a global signal, for example, from the shoot, is unable to account for the emerged LR at the generated curve. Secondly, in most studies nutrient conditions are kept homogeneous for the duration of root growth. For example, a homogeneous and sufficiently high nitrate availability leads to a uniformly sparse LR branching, while at lower (but not limiting) homogeneous levels the LR density increases (Figure 1c(i), [16]). Such a density response appears strategic, conserving resources under ideal nitrate conditions, but otherwise investing in foraging. It suggests a decision making at the root tip, with an increased likelihood to emerge at lower nitrate conditions.

In reality, however, many nutrients are almost exclusively present within heterogeneous patches [17], even after tillage [18], inducing much more complex root architectures. A classic experiment in barley [19], later replicated in *Arabidopsis* [20], shows that under heterogeneous soil conditions local LR emergence in high nitrate patches increases, while emergence is suppressed in the surrounding low nitrate regions (Figure 1c(ii)). From an optimisation point of view this is understandable, as investment in LRs then remains limited to the regions with highest nutrient availability. However, such an altered response compared to the homogeneous nutrient availability, with very different LR densities being generated at the same local nitrate level, requires either the prebranch site density to be modulated during primary axis growth when nutrient levels change, or requires LRP to communicate and modify their intrinsic behaviour. To our knowledge, the existence of priming modulations has not yet been shown.

Natural soil conditions are not only heterogeneous but also dynamically changing over time. Also when a high nitrate patch only becomes available for the root to exploit after it has grown through this region, increased emergence can be observed (Figure 1c(iii), [21]). This clearly requires LRP to locally sense the adjacent soil.

Finally, split-root experiments show the existence of also a systemic level of control (Figure 1c(iv)). When a root is split into two, each part immersed in different soil conditions, differential branching can be observed, again deviating from the expected pattern under homogeneous conditions [22]. This implies some form of systemic control. Roots even seem to anticipate temporal changes, as shown through split-root experiments in pea, with the

root system preferably investing in nutrient acquisition within temporally highly variable soil instead of soil with continuously low nutrient levels [23\*\*].

## A proposal: the rhizomer hypothesis

### The quantum of decision: the rhizomer

To reconcile all the empirical evidence presented above, we propose that the observed patterning is an emergent behaviour of a series of relatively autonomous multi-cellular modules. We define each module or rhizomer as a repeated developmental unit along the primary root axis, centred around a primed prebranch site that is competent to develop into a LR.

Although previously repeated modules and phytomers have been described for the plant shoot [3,4,24,25], the rhizomer is distinct in that it is not predetermined to branch, but rather processes incoming signalling to promote or suppress its developmental journey to LR emergence. A rhizomer is composed of cells from all tissues of the primary root within a lateral cross-section, so adjacent rhizomers do not have well-defined cellular boundaries. Their longitudinal length is roughly set by the oscillating genes that prime each rhizomer for competency to initiate and become a LRP. A rhizomer thus refers to a collective unit of cells that perceives and processes input signals to produce an effective decision regarding LR progression.

The backbone of the rhizomer hypothesis is a maintained competence for each initiated LRP to develop, which requires active communication with neighbours through both short-range and long-range signalling. The often antagonising input of these signals provides the rhizomer a non-linear decision-making capability. We consider that it is the decision to stop progression that spaces emerging LRs. When enough stimuli are provided, such as ample auxin, the rhizomer will branch, unless inhibited by even stronger signalling from its environment or neighbours. For example, LR promotion by auxin [11,26\*\*,27] can be inhibited by cytokinin [28]. We next discuss how the rhizomers could sense the local environment, relay information globally throughout the root system, and use intrinsic pathways, such as the auxin pathway, to make the branching decisions that allow for an adaptive response.

### The auxin pathway: decision-making logic

The rhizomer's progression from initiation to emergence is regulated by signalling pathways, primarily through auxin signalling [29]. This central pathway (Figure 2a) is modulated by local nutrient availability and signalling, which ultimately resolves into downstream developmental processes. It can simultaneously be modified by multiple nutrients, including iron, phosphate, sulphur, nitrogen and potassium [21,30,31\*\*], as well as non-nutritional cues, such as gravitropism, thigmotropism and root

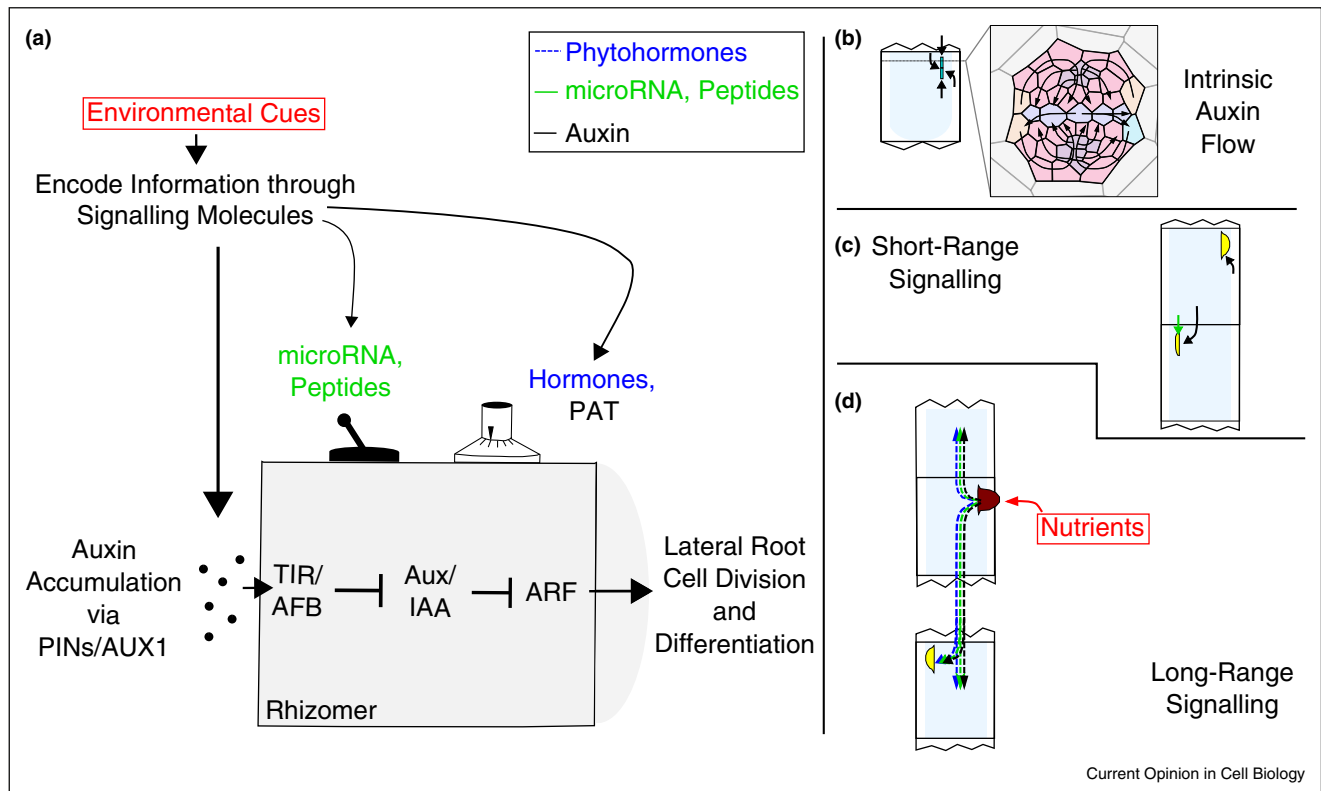
bending [14,15]. Such modifications occur either through direct interaction or through adjacent pathways. When an external cue is perceived, it is encoded into signalling molecules, directed to the appropriate cells within the rhizomer, and then decoded to inform decisions regarding developmental outputs, such as coordinated cell division and differentiation required for LR formation [29]. Encoded signalling molecules include small molecules such as phytohormones, miRNAs and peptides (Figure 2a). A signalling process within each rhizomer — a translation of perception into decision-making — assures progression from initiation to emergence. The auxin pathway provides numerous opportunities for signalling mechanisms to alter the decision. We propose the auxin pathway in its broadest sense to act on the individual rhizomer level as the 'decision-making algorithm' — constantly integrating intrinsic, local and global signals to determine if LR development should proceed.

### Integrative signalling networks: the intrinsic, local and global pathways

LR development requires the regulation of intrinsic, local and global signalling within and among the rhizomers. The intrinsic signalling within each rhizomer originates with the genes' oscillations and auxin modulations during priming (Figure 2b, [8,32]). Auxin is critical for LR initiation, as demonstrated in [14], and the rhizomer structure allows the individual capacities of several specialised cells to work together to direct auxin to the prebranch site at the xylem-pole pericycle cells [33\*\*], resulting in either a left or right LRP (Figure 2b). This local auxin maximum is mediated through auxin transporters [29] and symplastic diffusion via plasmodesmata [34,35\*].

The next signalling tier are the local signalling pathways that allow rhizomers to interact with their immediate neighbours and with the soil conditions (Figure 2c). Table 1 gives several examples of how environmental cues alter LR development, either by directly interacting with the auxin pathway, or through other signalling molecules. Direct interaction involves manipulation of proteins in the core auxin pathway (as shown in Figure 2a). Examples are the AUX1 response to iron [21] and the TIR1 response to phosphate [30]. Local stimuli can also promote auxin accumulation. The dual-transceptor NRT1.1 perceives nitrate and transports auxin, translating high local nitrate soil conditions into auxin accumulation [22,36]. Likewise, bending the root triggers local auxin accumulation [14,15], and it is hypothesised that this triggers initiation of the nearest primed site [8,37]. The concept of connected communicating rhizomers provides a framework for such a hypothesis. Phytohormones play a key role in the interaction between rhizomers. The flux and accumulation of auxin acts as an analog regulator, which can then be modified by other phytohormones. Cytokinin and strigolactone, for

Figure 2



The root system's architecture as an integrated response to the environment of a swarm of rhizomers. Signalling through the auxin pathway in the rhizomer. **(a)** The auxin pathway acts as a decision-making integrator, processing incoming information from the environment and other rhizomers. LR progression depends on accumulation of auxin in the rhizomer, resulting in LRP. External signals, such as other phytohormones, microRNA, peptides, and auxin itself, directly or indirectly affect the signalling cascade of the auxin pathway, adjusting the efficiency and progress of LR development. **(b–d)** Signalling can be grouped by proximity of the sender and receiver. **(b)** The rhizomer intrinsically directs all auxin flow towards the xylem-pole pericycle (XPP) cells, generating with the available auxin supply unique and mature LRP. Inset shows auxin flow through the tissues from the rhizomer's 'basin of attraction' to the XPP in a cross-section along the primary root axis. **(c)** Adjacent rhizomers participate in short-range signalling, shuttling auxin and small molecules through cell-to-cell transport and apoplastic diffusion. **(d)** Rhizomers also communicate with all other rhizomers and the shoot through long-range signalling — all forms of signalling molecules are transported through diffusion and vascular flow.

example, both modify the localisation of auxin transporters (PINs and AUX1) (Table 1, [28,38\*\*]). Bigger regulatory molecules such as miRNA and small regulatory peptides then act locally at the rhizomer level to fine-tune the signalling transduction pathway, effectively translating the analog signal into a digital output (Figure 2a,b). miRNA transcriptionally regulates the signalling pathway, while small regulatory peptides are secreted extracellularly, binding non-cell-autonomously to neighbouring cells' receptor kinases for downstream signalling [16,29,39–47].

The highest signalling tier is the global signalling. Small signalling molecules are also involved in this process, that is, the long-range interactions between rhizomers (Figure 2d). To communicate between non-adjacent rhizomers, global signalling takes place via phloem

and xylem, as well as through polar auxin transport. Phloem and xylem act as advective highways for the signalling molecules to travel throughout the root system and reach distant rhizomers (Table 1). The global signalling is part of the systemic control, the shoot also being a source of signalling molecules that can regulate rhizomers. In split-root experiments, in which each root is located within a different soil environment, signalling output of the roots has to be conveyed to the shoot, and the signalling responses of the shoot conveyed back to the entire root system, to be perceived by the rhizomers in each root, causing the soil environment of one root to affect the rhizomer decision making in the other root. Collectively, the intrinsic, local and global signalling pathways are integrated to allow for plastic modulation of the root architecture in response to the environment.

Table 1

Local and global signalling components			
Categories	Examples	Mode of action	References
<i>Local signalling</i>			
Phytohormone	Auxin	<ul style="list-style-type: none"> <li>• NRT1.1, nitrate transceptor (transporter and receptor) modulates auxin localisation through active transport at LRP in response to local nitrate availability.</li> <li>• Root bending mechanically induces redistribution of auxin transporters, PIN1 and AUX1, which mediates local auxin accumulation.</li> <li>• High iron availability promotes LR elongation by upregulating auxin import via AUX1 transporter.</li> <li>• Increased auxin receptor TIR1 expression in response to low phosphate availability alters auxin sensitivity, subsequently increases LR initiation and emergence.</li> </ul>	[36]
	Cytokinin	Negatively regulates local auxin accumulation by disrupting PIN localisation, even after addition of exogenous auxin.	[25,14]
	Strigolactone	Modifies PIN2 and AUX1 trafficking in response to low phosphorus, which reverses auxin flux from LRP to the epidermal tissues, leading to LR inhibition.	[21]
Micro RNA	miR393	Targets the auxin receptor AFB3 in response to nitrate.	[30]
	miR167	Targets the auxin response factors ARF6 and ARF8 in response to nitrate.	[28]
Peptide	CLE1, CLE3, CLE4, CLE7	Peptides produced at the pericycle locally inhibit LR emergence during nitrogen limitation by binding to a receptor on phloem companion cells.	[38**]
	CEP5	The peptide-encoding gene is nitrate-responsive and negatively regulated by auxin, which affects LR initiation.	[40]
<i>Global signalling</i>			
Phytohormone	Auxin	Auxin is transported from cell to cell via polar auxin transport and is loaded into phloem via the AUX1 transporter for long-distance transport.	[46,41]
	Cytokinin	The cytokinin biosynthesis gene IPT3 is upregulated under high nitrate conditions; subsequent high cytokinin levels in phloem communicate this nitrogen status from root to shoot.	[42]
Micro RNA		Small RNAs are shown to be mobile and transported through the whole plant, suggesting involvement in global signalling regulation.	[43]
Peptide	CEPs	Nitrogen-responsive CEP peptides isolated from xylem sap were shown to systemically regulate LR development under nitrogen limitation.	[44]

## Conclusion

Considering how root architecture responds to homogeneous and heterogeneous environments requires us to acknowledge both non-local and non-centralised processes, somehow intimately coupling, both spatially and temporally, LR priming, initiation and emergence. To embrace both the recent molecular findings that the root tip exerts a pre-patterning influence on final rhizotaxy and that local perception of the environment and global signals funnel into LR developmental pathways in a determinant manner, we propose to view the root as a string of interacting rhizomers. Root foraging can then be regarded as the collective behaviour of such rhizomers that progress through or halt at LR developmental stages due to intrinsic, local and long-distance signalling.

## Acknowledgements

This work was supported by the Swarm-Organ Consortium through European Union Commission [FP7-ICT-2011-9-601062] and the UK Biological and Biotechnology Research Council [BB/J004553/1]. We thank Susan Duncan, Stan Maréc and the Swarm-Organ Consortium for fruitful discussions, Juan Perianez-Rodriguez and Miguel A. Moreno-Risueno for their courtesy in providing image Fig. 1b, and the reviewers for valuable feedback.

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