Trends in Plant Science

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Spotlight

Low Phosphate Puts Auxin in the Root Hairs

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The molecular changes that allow plant roots to response to low phosphate levels are poorly understood. A series of three papers investigate this phenomenon and reveal which components of the auxin response are key for transmitting the phosphate signal into changes in root hair phenotypes.

Phosphate is a macronutrient essential for plant growth. It has long been known that alterations in the amount of available phosphate can have a significant effect on root architecture and on root hair elongation via the auxin response [1–3]. This is the starting point for a series of three manuscripts published back-to-back-to-back in *Nature Communications*, which investigate the molecular events that lead to the root hair phenotypes that result from growth in low-phosphate conditions. Although many of the molecular players described in this research have been previously characterised, these manuscripts place them in a stepwise manner to provide a mechanistic framework that links phosphate levels with auxin signalling.

Two of these studies originate from the labs of Malcolm Bennett and Ranjan Swarup in the Department of Plant Sciences at the University of Nottingham, and provide evidence, if it were needed, for the continued utility of model organisms to understand crop phenotypes. Somewhat unusually, this research began in rice (*Oryza sativa*) and then moved to *Arabidopsis* (*Arabidopsis thaliana*), where a molecular framework could be more easily defined.

The AUX1 protein was characterised over 20 years ago as an auxin influx carrier involved in a range of root-specific phenotypes, including in the response to gravity and during lateral root and root hair formation [4]. In the first of these new papers, Giri *et al.* [5] use MicroCT imaging to characterise root architecture in rice *osaux1* mutants, discovering that they form a shallower root angle, resulting in a higher proportion of roots remaining in the top soil. As phosphate accumulates in this area of the soil, intuitively the authors thought that this phenotype might provide a growth advantage. However, they found that phosphate acquisition was not improved, due to a reduction in root hair elongation in response to low phosphate. This root hair phenotype overrides any beneficial effect gained from the root positioning. The limited molecular tools available in rice were used to confirm that the response to low phosphate involved the plant hormone auxin. Although not included in this manuscript, in future it will be important to understand whether the gene expression changes that occur in rice roots are similar to those in *Arabidopsis*, or whether a different set of auxin-regulated genes respond to low phosphate in this crop plant (Figure 1).

In contrast to the usual pathway of discovery from model to crops, the authors then turned back to *Arabidopsis* with the aim of better understanding the relationship between auxin and low phosphate at the molecular level. Using *Arabidopsis*, Bhosale *et al.* [6] were able to discover which previously characterised members of the auxin-signalling pathway were involved in transmission of a signal that begins with the perception of a low-phosphate environment and ends with changes in root hair phenotypes.

Previous studies [1] suggested that low-phosphate conditions might cause an increase in auxin biosynthesis in the root and Bhosale *et al.* [6] extended this analysis by using gas chromatography–mass spectrometry to show the increase is specific to the root hair region. In their remaining experiments, Bhosale *et al*. [6] use genetic analysis and interrogation of gene expression data to characterise the role played by auxin biosynthesis, transport, and signalling toward the final phenotype. Both the TRYPTOPHAN AMINOTRANSFERASE OF ARABIDOPSIS1 (TAA1) and DIOXYGENASE FOR AUXIN OXIDATION1 (DAO1) genes affect auxin homeostasis and the expression of both genes is upregulated in the root tip in response to growth on low phosphate. DAO1 is involved in auxin degradation, yet the weak phenotypes in *dao1* loss-of-function mutants led the authors to question of the importance of this process during the root hair response. Through additional genetic analysis it would be interesting to learn whether the author hypothesis is correct or whether auxin degradation is indeed important in this process, where the DAO1 gene acts redundantly with other signalling components that control auxin homeostasis.

Bhosale *et al.* [6] demonstrate that expression of the *Arabidopsis* AUX1 gene in only the lateral root cap and epidermal cell layers is sufficient to transport newly synthesized auxin from the root tip to nascent root hair cells. In addition, they show the auxin response factor ARF19 is required for an increase in expression of the bHLH transcription factors RSL2 and RSL4: genes that are known to be required for root hair elongation. Losing the activity of any of these components reduces the amount of root hair elongation in response to low phosphate. There are many other known components of the auxin signalling pathway, yet in this manuscript the authors only focus on a small number of these. It remains to be seen whether this signal is indeed transmitted by a small set of proteins or whether other molecular players are involved that were not investigated in this manuscript.

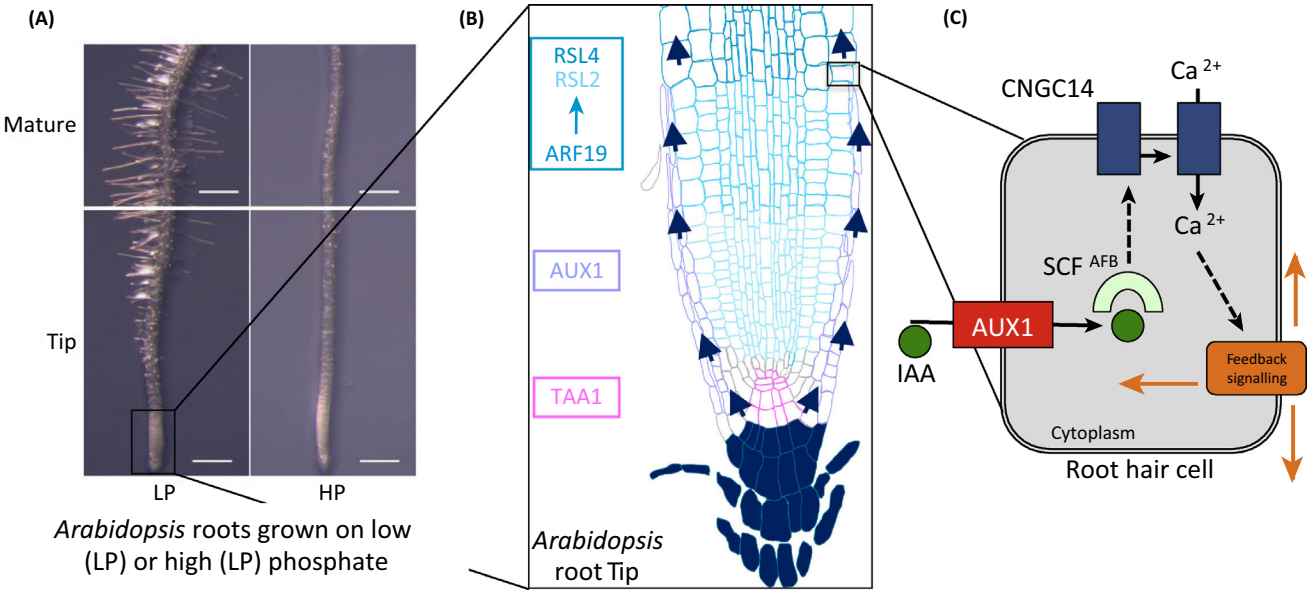

Figure 1. Changes in Root Architecture under Low-Phosphate Conditions Are Linked to Auxin Signalling. (A) *Arabidopsis* roots grown on low or high concentrations of phosphate show different amounts of root hair elongation (adapted from [6]). (B) In low-phosphate conditions, auxin is synthesised in the root tip and transported by AUX1 into root hair cells, where elongation is promoted by the expression of the ARF19, RSI2, and RSL4 genes (adapted from [6]). (C) Auxin (IAA) is transported into root hair cells by AUX1, where a proposed novel rapid activity of SCF^{AFB} increases calcium influx via CNGC14. Increased cytoplasmic Ca^{2+} causes feedback signalling both in a cell autonomous and non-autonomous manner (adapted from [7]).

The final paper of this trio focuses on the effect of auxin-induced membrane depolarisation on root hair cell elongation. This research was led by Julian Dindas from the lab of Rainer Hedrich at the University of Würzburg and is linked to the other manuscripts through their collaboration with Malcolm Bennett [7]. In a set of technically impressive experiments Dindas *et al.* [7] attach microelectrodes to bulging root hairs and observe an auxin-dose dependent membrane depolarisation that requires the activity of the AUX1 protein. Membrane depolarisation results in calcium influx and it has been previously shown that auxin causes this response through the activity of the CYCLIC NUCLEOTIDE-GATED CHANNEL 14 (CNGC14) protein [8].

In an exciting set of findings, Dindas *et al*. link auxin-induced membrane depolarisation with the TIR1/AFB auxin-signalling module [7]. This result sits apart from the phosphate–auxin narrative that leads the other manuscripts. However, it is extremely interesting, as it shows that the $SCF^{TIR1/AFB}$-signalling module is involved in rapid auxin-induced membrane depolarisation and subsequent calcium influx mediated by CNGC14. The speed of this response most likely rules out the involvement of gene expression changes via the action of Auxin Response Factor (ARF) proteins. Thus the authors propose that $SCF^{TIR1/AFB}$ might contribute to the degradation of an unknown membrane-localised catalytic enzyme, although they do not provide an explanation as to how this degradation, which is usually confined to the nucleus, might occur. In addition, the authors do not link to the Bhosale *et al.* [6] manuscript in order to provide an explanation as to how this finding might integrate with the role of the ARF19 protein in control of root hair elongation.

Following publication of these three manuscripts, further evidence emerged regarding the role of $SCF^{TIR1/AFB}$ in an auxin response that does not require *de novo* gene expression. Fendrych *et al*. [9] developed a novel microfluidic-based experimental system to detect extremely rapid changes in root growth rate following applications of external auxin. They showed that AUX1 is critical for this rapid response and their work led them to conclude that this was through an increase in auxin cellular accumulation. Whereas the accumulation of cellular auxin is clearly linked to auxin-induced membrane polarisation, Fendrych *et al.* [9] did not use their system to test whether this response is critical for the rapid auxin response. Subsequently they used an approach that utilised a recently developed synthetic *in planta* TIR1-IAA interaction [10] to demonstrate that the $SCF^{TIR1/AFB}$ module was indeed necessary for rapid auxin-induced changes in root growth.

By showing that root hair membrane depolarisation occurs more rapidly in

roots that had been 'primed' by growth on low-phosphate concentrations, Dindas *et al.* [7] link their findings to the other two *Nature Communications* manuscripts. The authors contend that the evidence from the associated studies shows that these low-phosphate growth conditions will increase the amount of AUX1 protein, therefore enabling a faster response to externally added auxin (Figure 1).

Finally, Dindas *et al.* [7] combine a calcium sensor and auxin reporter to demonstrate that auxin-induced calcium influx in a single root hair cell sets off a chain of similar responses in adjacent cells. By simultaneously evaluating the expression of the auxin-responsive DII::VENUS reporter they show that the calcium flux corresponds to increased auxin responses in these adjacent cells. This might provide a potential mechanism whereby minute changes in local phosphate concentration provoke a phenotypic response in surrounding cells in order to maximise the uptake of all available nutrients.

In future it will be informative to link the findings of both Dindas *et al.* [7] and Fendrych *et al.* [9] to understand how membrane depolarisation, calcium signalling, auxin accumulation, and the activities of CNGC14 and $SCF^{TIR1/AFB}$ are linked. It will be particularly revealing to understand how these responses act within the whole cell context with regard to the localisation of auxin-signalling components to the plasma membrane, cytosol, or nucleus.

The extended time between submission and acceptance (>1 year), as well as the multiple review cycles, clearly demonstrates the challenge of getting three linked manuscripts into a position where they are each ready for publication. Taken alone, the majority of the findings presented in these papers are not novel, such as the relationship between phosphate, auxin, and root hair elongation, and the identity of auxin-signalling components, or the link between auxin, membrane depolarisation, and calcium influx. However, together these papers provide a narrative that links an important environmental response to the fine detail of plasma membrane dynamics and auxin-mediated gene expression. In addition, Dindas *et al*. [7] provide early evidence for a novel mechanism through which the activity of $SCF^{TIR1AFB}$ might impact rapid auxin signalling, a discovery that has been confirmed in the more recent Fendrych *et al.* [9] publication. Given the broad importance of auxin signalling in plant development, these findings should be of interest to a wide range of plant cell biologists.

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