Strapline: **Rapid auxin responses**

Title: **It starts with TIRs**

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Standfirst: The canonical auxin receptor complex mediates gene expression, but it is also necessary for responses far too rapid to be mediated by transcription. An innovative setup that uses advanced microscopy and microfluidics can record auxin-induced changes within 30 seconds during root growth.

Main text (811 words)

The discovery of Transport Inhibitor Response1 (TIR1) and Auxin signalling F-Box proteins (AFBs) as auxin receptors was a landmark in plant biology, identifying not only the binding site for this crucial hormone, but also confirming the primary mechanism through which auxin controls gene expression[1-3]. It is now becoming increasingly clear that the TIR/AFBs are also necessary for responses initiated within less than a minute, making it likely that TIR1/AFBs control both transcriptional and non-transcriptional aspects of auxin signalling.

Two recent reports use different, but complementary approaches to report that *Arabidopsis* primary root elongation growth[4] and ion fluxes across root hair plasma membranes[5] show responses to exogenous auxin that are measurable within seconds, and are switched off almost as rapidly. Such response times appear far too fast to involve changes in transcription. Alternative receptor candidates have been a part of the history of auxin signalling[6], so it is an interesting twist that we now have to explain how the TIR1/AFB receptors can act in more than one way.

Fendrych et al. use a specially-developed microscope[7] and microfluidic chip[8] to watch primary roots grow in real time. The fluidics allowed precise control of the arrival and departure of auxin treatments and, hence, analysis of response times. Remarkably, elongation growth was inhibited within 30s of auxin reaching the root (Fig. 1). Perhaps even more remarkably, growth was resumed within 2 minutes of auxin removal which suggests extremely active mechanisms for homeostasis management and acute responsivity of the system to changing auxin stimuli. The 50% inhibition constant (IC_{50}) for indole-3-acetic acid (IAA) was 1.44 nM demonstrating exquisite sensitivity of auxin sensing in the root.

Similarly, rapid response times were also reported recently in *Arabidopsis* root hairs for auxin-induced plasma membrane depolarisation and rises in intracellular calcium concentration[5]. In this case the auxin doses used were somewhat higher, generally 10 µM (vs 10 nM for elongation growth) with correspondingly higher Michaelis constants (Km), but the use of auxin analogues demonstrated auxin specificity.

Having established assays for rapid responses, both groups asked whether the site of perception was inside or outside the cell. In agreement with earlier work showing auxin-dependent changes in plasma membrane ion fluxes[9], both found that their responses were

dependent on the auxin uptake transporter AUXIN1 (AUX1), implying that the receptor was intracellular.

Inhibition of primary root growth by auxin is a well-studied response and known to be linked to TIR1/AFB-mediated ubiquitination and control of transcription[10]. Therefore, despite the rapidity of the responses, both groups tested for attenuation of their assay outputs in *tir1-1/afb2-1/afb3-1* triple mutants. This loss of half of the receptors caused significant reductions in both cases, implying that the TIR1/AFBs are necessary elements of the rapid, non-transcriptional response pathway - as well as that for transcriptional control. Each group supplemented their mutant data with inhibition experiments to confirm the role of TIR1/AFBs: adding the anti-auxin auxinole[5,11], or using a new synthetic biology tool, a re-engineered receptor (known as concave-TIR1 due to designed mutation of the auxin-binding pocket) and its matching synthetic ligand known as convex-IAA[4,12]. Convex-IAA is only specific for concave TIR1 and not otherwise an active auxin; and this version of TIR1 is otherwise unaltered enabling it to engage with all its normal substrates, the family of transcriptional regulators known as AUX/IAAs. An Arabidopsis line transformed to express concave-TIR1 responded to convex-IAA with rapid growth inhibition similar to that seen with IAA and wild type plants. This elegant use of novel synthetic biology tools confirms the involvement of TIR1/AFBs in rapid growth beyond reasonable doubt.

Having established that the plants read intracellular auxin for rapid responses using TIR1/AFBs, many questions pose themselves. How does TIR1 work in this alternative mode; is the same binding pocket used; is TIR1 in the nucleus for rapid responses, etc? Given the link with plasma membrane ion fluxes, the Cyclic Nucleotide Gated Channel protein CNGC14 and apoplastic pH change, Dindas et al. proposed that some TIR1 migrates into the cytoplasm and some remains in the nucleus for slow responses[5]. Fendrych et al. speculate that some ubiquitinated AUX/IAAs escape the proteasome and move out of the nucleus to mediate rapid responses[4]. A further possibility is that auxin binding displaces an unknown TIR1 interactor prior to promoting AUX/IAA binding.

The history of auxin signalling is littered with reports of responses considered too rapid to be mediated by transcriptional control[3]. The mechanisms behind such non-transcriptional signalling have remained obscure despite some of the principal players being consistently implicated. Amongst these are cytosolic calcium concentration, the movement of protons across the plasma membrane, associated changes in membrane potential, and apoplastic pH. One of the most reassuring features of this root growth study[4] is that the response measured is of a whole, uncompromised plant made possible by advances in microscopy platforms and microfluidics. There remains a mechanistic gap between TIR1 and activities in the apoplast, but that gap is narrowing.

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Figure 1: **Microfluidics and confocal microscopy to study very fast responses to auxin**.
A novel experimental setup is used to observe primary root growth. It includes live imaging with confocal microscopy, coupled with microfluidics to supply the roots with chemicals or wash them away in seconds. A fast response to auxin in wild-type is the inhibition of primary root growth. Mutant genetic backgrounds or transgenic plants containing synthetic biology constructs are used to show that these fast responses depends on auxin influx transporter AUX1 and the TIR1/AFBs auxin receptors.