The authors argue that further measurements with improved precision will allow tunnelling to be characterized more fully. Such measurements will require X-ray pulses significantly shorter than those of a few hundred attoseconds that are presently available. Once this hurdle has been surmounted, the tunnelling method could be used to probe how electrons behave in a wide range of atomic and molecular systems with attosecond precision.

In the meantime, Uiberacker *et al.* turn their attention[1] to the significantly slower processes associated with the decay of excited states in the xenon ions Xe^{2+} and Xe^{3+}. Using their laser-field tunnelling method to produce Xe^{4+} ions, they have been able to measure cascaded population transfer processes among the electron states of ions that have lifetimes of as little as 6 femtoseconds (6×10^{-15} s). They thus already show the potential of their method to extend and increase the precision of findings established in earlier spectroscopic studies. ■

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PLANT BIOLOGY

Sticking with auxin

Tom Guilfoyle

Auxin is one of the main agents that regulate plant growth and development. Intricate crystallographic studies reveal how this hormone acts as a 'molecular glue' in mediating substrate–receptor interactions.

Research into plant hormones has been coming on apace in the past few years. The receptors for these hormones that have been identified, including that for auxin, have turned out to be surprisingly different from the receptors for animal hormones[1]. On page 640 of this issue, Tan *et al.*[2] take the story further — their first-of-a-kind crystallographic studies provide more revelations about auxin perception and the auxin receptor.

In 2005, a receptor for auxin (or indole-3-acetic acid, IAA) was identified as the F-box protein TIR1, short for 'transport inhibitor response 1'; TIR1 is a component of a cellular protein complex known as SCF^{TIR1} (refs 3, 4). The substrates for TIR1, Aux/IAA repressors, are recruited to the receptor in an auxin-dependent manner and, after binding to TIR1, are degraded. Identification of the TIR1 receptor suggested that auxin perception and the signalling pathway to auxin-regulated gene expression was direct and simple, but it left various questions. Tan *et al.*[2] now describe crystal structures of a TIR1 complex that reveal how auxins fit into a surface pocket of TIR1 and enhance the binding of Aux/IAA repressors to TIR1.

Auxin-regulated gene expression triggers most of the processes controlled by this plant hormone. Many auxin-induced genes are regulated by the interplay of two classes of gene-transcription factors, auxin-response factors (ARFs) and the Aux/IAA repressors[5] (Fig. 1). ARFs bind to auxin-response promoter elements in auxin-response genes. When auxin concentrations are below a threshold level, Aux/IAA repressors associate with ARF activators and repress the expression of these genes. Conversely, higher levels of auxin lead to destruction of the Aux/IAA repressors, and to activation of the genes[6,7].

Aux/IAA repressors contain four conserved domains, and one of these, domain II, is responsible for the instability of these proteins (Fig. 2a, overleaf). Domain II has a hallmark GWPPV amino-acid motif that is recognized by TIR1 in the SCF^{TIR1} complex[8,9]. IAA binds to TIR1 to enhance the recruitment of the motif to TIR1, as do two synthetic auxins — 1-naphthalene acetic acid (1-NAA) and 2,4-dichlorophenoxy-acetic acid (2,4-D, which is used as a herbicide) — but with different affinities (Fig. 2b).

Tan *et al.*[2] expressed TIR1 complexed with ASK1 (a SCF^{TIR1} adaptor) in a baculovirus–insect system. After demonstrating that auxin enhanced the binding of an Aux/IAA protein to the complex, they obtained crystal structures for the complex alone and for complexes bound to IAA and the two synthetic auxins along with an Aux/IAA peptide containing the GWPPV motif (Fig. 2). The crystal structures showed that the TIR1–ASK1 complex had a mushroom shape, with the leucine-rich-repeat domain of TIR1 forming the cap, and the F-box of TIR1 along with ASK1 forming the stem. A pocket on the top of the TIR1 leucine-rich-repeat domain functions in both auxin binding and substrate recruitment.

It turns out that auxin binds to the bottom of the pocket in a 'promiscuous' binding site that tolerates moderately different planar ring structures (that is, natural and synthetic auxins). The Aux/IAA peptide binds in close proximity to the auxin-binding site in the upper part of the pocket. The GWPPV motif is packed directly against auxin and covers the auxin-binding site. This is thought to trap auxin in the binding pocket until the Aux/IAA peptide is released and moved along the degradation pathway. The crystal structure also revealed an unexpected moiety in TIR1, which turned out to be a tightly bound inositol hexakisphosphate ($InsP_6$) molecule. $InsP_6$ functions in many

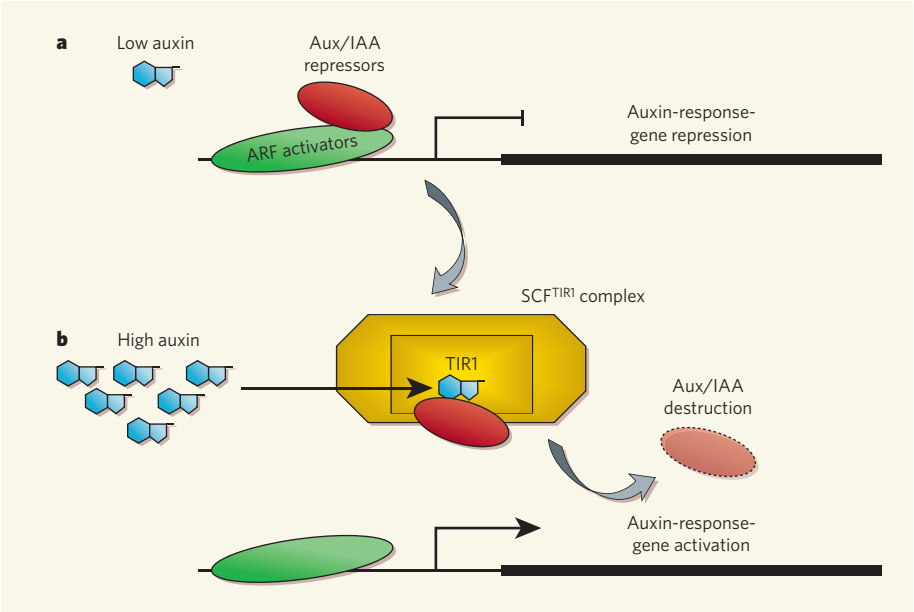

Figure 1 | The auxin signalling pathway. ARF activators bind to auxin-response elements in promoters of auxin-response genes. **a,** When auxin concentrations are low, Aux/IAA repressors associate with the ARF activators (via domains III and IV, see Fig. 2) and repress expression of the genes. **b,** When auxin concentrations increase, auxin binds to the TIR1 receptor in the SCF^{TIR1} complex, leading to recruitment of the Aux/IAA repressors to TIR1. Once recruited to the SCF^{TIR1} complex, the repressors enter a pathway that leads to their destruction and the subsequent activation of the auxin-response genes.

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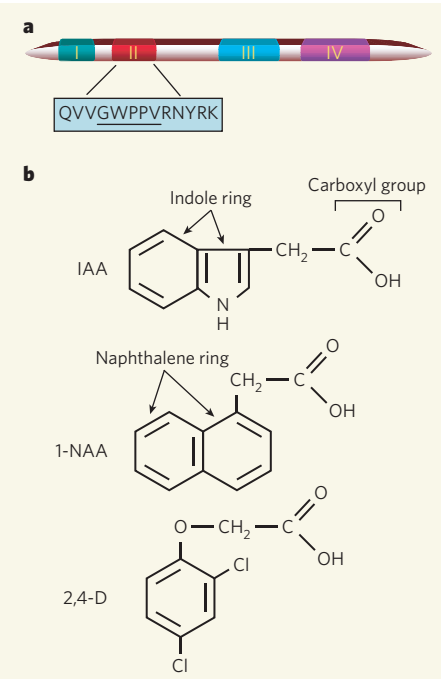

Figure 2 | The main molecular players in the work of Tan *et al.*[2]. **a**, Depiction of the four conserved domains of Aux/IAA repressors. The synthetic peptide from domain II is sufficient for targeting Aux/IAA to the TIR1 auxin receptor, the core sequence being GWPPV (G, glycine; W, tryptophan; P, proline; V, valine). **b**, The three auxin ligands that the authors crystallized in association with the TIR1 auxin receptor. IAA itself, and the synthetic auxins 1-NAA and 2,4-D, bind to a 'promiscuous' cavity in the receptor with different affinities, but all of them stabilize the interaction between the Aux/IAA repressor and the receptor.

cellular processes, including acting as a phosphate reservoir in plants. Its association with TIR1 appears to be essential for auxin binding and function of the receptor.

The authors compared TIR1 structures that had IAA or the two synthetic auxins in the ligand binding site. IAA binds to TIR1 with the greatest affinity of the three auxins, and binding involves its side-chain carboxyl group as well as its indole ring (Fig. 2b). The synthetic auxins bind to TIR1 in a manner similar to IAA, but with affinities determined by how effectively their ring structures fit into and interact with the promiscuous cavity of the receptor. The auxin herbicide 2,4-D has the weakest affinity for TIR1, having the smallest ring and the least amount of surface contact with the cavity of the receptor. Although 1-NAA has the largest ring and the most surface contact with the cavity, it has only an intermediate affinity for TIR1. The smaller indole ring of IAA does not fit the cavity as well as the naphthalene ring of 1-NAA, but it makes an additional contact with the receptor, which increases its affinity.

Interestingly, the binding of auxin does not induce significant conformational changes in TIR1. Instead, auxin enhances the binding of Aux/IAA substrate to TIR1 by occupying a cavity between substrate and receptor, thus forming a continuous hydrophobic core among ligand, substrate and receptor. The authors characterize this as a 'molecular glue' that effectively strengthens the binding of the substrate to the receptor.

Tan *et al.*[2] have provided the first detailed structure of a plant hormone receptor, but further questions are the inevitable upshot. Do plant hormones such as jasmonic acid bind to and function as molecular glue in TIR1-related F-box proteins[10]? Are there TIR1-like proteins in organisms besides plants that function as hormone receptors or small-molecule sensors? And how interconnected is InsP6 metabolism with auxin signalling and TIR1 activity? Several labs will soon be hard at work in taking these next steps. ■

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EVOLUTIONARY BIOLOGY

Born-again hagfishes

Philippe Janvier

The strange, slimy creatures called hagfishes are of abiding interest to students of vertebrate evolution: just where do they fit in? Investigations of hagfish development take the story forward.

Hagfishes are almost blind, cartilaginous, eel-shaped, marine vertebrates and, most notably, they lack jaws. Their relationships to other major living vertebrate groups — the similarly jawless lampreys, and the jawed vertebrates — remain contentious, and one avenue of investigation is to look to embryonic development for further information. Alas, hagfish embryos that are suitable for such studies have been desperately rare. On page 672 of this issue[1], however, Ota and colleagues describe the first early embryos from hagfishes to have been found since 1930, and report their studies on gene-expression patterns in them*.

Hagfishes are occasional scavengers, and are frequently found inside dead fish. So Linnaeus[2] classified them among the 'intestinal worms', although he noticed that they share some characteristics with lampreys, and thus with vertebrates. They then became classified with lampreys as cyclostomes ('rounded mouth') because both possess a jawless mouth armed with retractable horny teeth, and gills enclosed in pouches. Living vertebrates thus fell into two major groups, the cyclostomes and the jawed vertebrates (or gnathostomes), which remained the received view for more than 170 years.

However, biologists progressively noticed that hagfish anatomy and physiology were in many respects more 'simple' or 'primitive' than those of lampreys and jawed vertebrates. Unlike these two groups, hagfishes lack vertebrae, heart innervation, eye lenses, lymphoid tissues, a perfected adaptive immune response, and many other classical vertebrate characters. In these respects, then, they resemble non-vertebrate chordates such as amphioxus or sea-squirts. That meant that there are two possibilities (Fig. 1). First, that hagfishes are 'degenerate' cyclostomes — that is, they have lost several vertebrate characters that lampreys and jawed vertebrates retain (unless independently acquired, which is unlikely). Second, that they are actually the most primitive vertebrates, and so are the 'sister group' of all other vertebrates[3]. Phylogenies based on DNA and RNA sequencing generally support the first hypothesis, but remain ambiguous[4,5].

Palaeontology sometimes settles such conflicts. But it is powerless in this case, because the earliest (300-million-year-old) hagfishes, preserved as soft-tissue imprints, are very similar to living ones. Moreover, the distribution of lampreys and gnathostomes through time suggests that hagfishes had a 'ghost range' of between 50 million and 170 million years during which they were apparently present, but are unrecorded[6]. In such cases, embryonic development may provide clues, on the assumption that early embryos may mirror ancestral conditions. But hagfish eggs found on the sea floor generally contain no visible embryo, probably because their development is very slow and early embryos may be missed[1]. Out of the 150 fertilized eggs found between 1896 and 1930, only a few embryos could be studied, but the techniques then in use did not allow detailed description.

However, among the embryonic hagfish

*This article and the paper concerned[1] were published online on 18 March 2007.