Eva Zažímalová · Jan Petrášek
Eva Benková *Editors*

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ISBN 978-3-7091-1525-1 ISBN 978-3-7091-1526-8 (eBook)
DOI 10.1007/978-3-7091-1526-8
Springer Wien Heidelberg New York Dordrecht London

Library of Congress Control Number: 2014943106

Printed on acid-free paper

Springer is part of Springer Science+Business Media (www.springer.com)

What Is Auxin? How It Operates?

Many articles dealing with plant growth and development start with the "auxin mantra", such as: Auxin is involved in control of many developmental processes in plants.

When in 1881 Charles Darwin and his son Francis examined coleoptiles exposed to unidirectional light, and proposed the existence of a signalling molecule directing their bending, they might have not been fully aware of enormous significance of their discovery for understanding the key principles governing plant growth and development. Since then the mysterious signalling molecule was identified, named auxin, and an immense number of observations confirmed a crucial importance of this tiny compound throughout life of any plant. Besides its for a long time known function in regulation of organ bending in response to light and gravity, auxin was revealed to mediate growth reactions of plants to current environmental conditions in general, and on top of that to control also genetically pre-programmed physiological processes such as embryogenesis, and initiation and formation of diverse organs including flowers, leaves, shoots, roots, and ovules. However, in spite of tremendous progress in the auxin research in last decades, it is still not fully understood how auxin operates and how it can regulate so many and so different processes. So, in spite of years of intensive research bringing much essential information, auxin still remains rather enigmatic.

In this book, respected scientists—experts in different fields of "auxinology"—summarize recent progress in understanding of how auxin operates to control and coordinate plant development. In 18 chapters various aspects of auxin biology focusing on auxin metabolism, transport, signalling, and principles of auxin-regulated plant organogenesis, tropic responses, as well as other interactions with environment are reviewed and future perspectives are outlined.

We hope this compact contemporary overview on the enigma called auxin will inspire new fresh research ideas to address remaining auxin challenges.

Prague and Klosterneuburg
May 2014

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Part I
Auxin: Definition; Metabolism, Transport and Signalling

Chapter 1
The Auxin Question: A Philosophical Overview

Tom Bennett and Ottoline Leyser

Abstract In this opening chapter, we ruminate upon "the auxin question": what is auxin? It is a seemingly simple question with no simple answer. We firstly try to provide a philosophical framework for understanding the question itself. We then discuss some possible answers to the question, and examine how these answers might help to drive the future direction of auxin research. We also offer some speculations on the evolution of auxin, and how such a simple molecule may have accrued such diverse functions.

1 Introduction: The Auxin Question

At a recent conference, the following conversation between two imaginary auxin researchers was overheard:

A: "Auxin does everything."
B: "Yes, but what is auxin?"
A: "I have to go now."

As an author, the simultaneous excitement and terror caused by an apparently open-ended brief is difficult to match. For this opening chapter, we were asked simply to reflect upon the question "what is auxin?" It seems innocuous as it lies there on the page, just three words, twelve characters—and yet, like a coiled serpent, it is *very* much longer than it appears and should be approached *very* carefully. The longer the question is contemplated, the harder it becomes to give a straight answer; it is the sort of metaphysical question that might drive one to

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E. Zažímalová et al. (eds.), *Auxin and Its Role in Plant Development*,
DOI 10.1007/978-3-7091-1526-8_1,

madness. It would be pleasing if there was a simple answer at least to begin at, but even taken at its most literal, the question does not have a definitive answer. Perhaps it is not possible to give a perfectly clear, unifying answer, only dim reflections on individual facets of the question; then again, perhaps the answer *is* simple, but we do not really understand the question we are asking.

What follows is our attempt to provide some kind of answer to this question, and to the deeper question that is wrapped wolf-like within the clothing of the first; how does such a simple molecule have such incredibly various effects? We also offer some speculation as to *why* auxin is so complicated; how and why has the system evolved in this way? Forced for once to confront these questions, rather than skirt round them, our own perspectives on auxin have shifted, and as much as anything else, we are critiquing our own previous (and perhaps current) misconceptions. This is inevitably a personal reflection on auxin, and we claim no great authority to answer "the auxin question", but if it is enough to stimulate debate as to the nature of "the auxin answer", then it will have served its purpose.

2 A Conceptual Framework

Conceptual frameworks can be very useful in driving science forward, but equally, they can hinder progress if the framework does not correctly formulate the question being addressed. Although it is not quite the sense in which Kuhn (1962) used the term, we might reasonably describe the current "regulator of development" model of auxin action as a paradigm. Over the last 80 years, there has been a repeated shift in the paradigm of auxin, as new discoveries (in many fields) have challenged existing theories (reviewed in Abel and Theologis 2010; see also Estelle 2009). We might persuade ourselves that the recent explosion in our knowledge of auxin means that our paradigm is more robust than in past generations—that we are now nearer "*the answer*"—but such optimistic thinking pervades any paradigm (Kuhn 1962). Certainly, we know more, but the shallowness of our *understanding* is amply demonstrated by the fact that we can still legitimately ask "what is auxin?" and not give a clear answer. It is inevitable that the next generation of auxin scientists will look back on many of our theories and smile at aspects of our misguided logic, just as we view many of the ideas of previous generations.

A conceptual framework is at its most useful when it allows us to synthesise previously unconnected ideas, or to reconcile previously problematic observations. They are also undoubtedly useful in the communication of science, particularly to researchers in other fields. However, they begin to be counter-productive when they become a "truth" in their own right, to be proved and defended. Then, effort is diverted into experiments that seek to confirm rather than test a model ("confirmation bias"), or seek to smooth over its inconsistencies, instead of striving for greater understanding of the actual biological problem. To pick an example from plant science, the ABC model of flower development was, and still is, an excellent framework for understanding mutant phenotypes of *Arabidopsis* and *Antirrhinum*

flowers; however, arguably for some it had a constraining effect, with too much effort spent in "perfecting" it, despite its inconsistencies and shortcomings, rather than using it as a starting point for a deeper understanding of flower development (e.g. Gutierrez-Cortines and Davies 2000).

It is inconceivable that we can answer "the auxin question" without some kind of conceptual framework, but what form should this framework take? It seems fairly clear that our current theories are insufficient to explain the bewildering mass of data that we have generated. Partly this is a function of the current funding-dissemination structure of science; we conceptualise our research as easy-to-digest, easy-to-sell, "provable" theories, which are by necessity self-limiting. Can we instead, as a community, establish a robust concept of auxin which brings together all our knowledge, which drives our research forwards, and leads to a genuinely deeper understanding of auxin and its role in plant biology? Such a framework must be only that; a flexible set of ideas, constantly modified as our knowledge increases; we must not cling on to ideas simply because they are convenient or pleasingly tidy.

So, what is auxin? Where do we begin?

3 Auxin Is Indole-3-Acetic Acid

Our philosophical struggle to understand auxin begins even with its chemical identity. At least we can reassure ourselves that it was ever thus; the original attempts at purifying auxin identified two auxins (auxin-A and auxin-B) that were in all probability non-existent (Wildman 1997); only somewhat later was an actual auxin identified, namely indole-3-acetic acid (IAA) (Kogl et al. 1934). Nevertheless, the idea of multiple "auxins" became firmly entrenched, no doubt helped by the fact that the second plant hormone to be identified, cytokinin, really does have multiple active forms. Furthermore, the definition of auxin was for a long time based on bioassays rather than an explicit chemical description (Abel and Theologis 2010; Simon and Petrášek 2011). This means that a large number of chemicals showing auxinic activity are described as auxins (or anti-auxins), even though most are not naturally occurring. This has led to the terms auxin and IAA being used non-synonymously. To an extent, this might not matter too much, and the continued use of vague terminology does pleasingly hark back to a golden age in plant research. However, after nearly a century, we really ought finally to define what we mean by auxin. It is remarkable that despite the revolutionary advances in our understanding, we persist with such a hazy definition of a molecule that is so important it warrants a whole book.

The first step in a strict definition must be to reject as an "auxin" any molecule that does not occur naturally in plants or green algae. Standard biological terminology would be to describe these molecules, such as naphthalene-1-acetic acid (NAA) and 2,4-dichlorophenoxyacetic acid (2,4-D) as auxin analogues (or auxin mimics, maybe even auxin agonists). Based on our current knowledge, this leaves us with four endogenous molecules that have been shown to exhibit auxinic activity

to some degree: IAA itself, indole-3-butyric acid (IBA), 4-chloro-indole-3-acetic acid (4-Cl-IAA) and phenyl acetic acid (PAA). In the case of IBA, there is relatively little evidence that it has inherent activity, but it is readily converted to IAA and may therefore represent a storage form of IAA (reviewed in Simon and Petrášek 2011). Other storage/degradation forms of IAA (e.g. IAA-lysine and IAA-leucine) are not referred to as "auxins", so there is no pressing reason to describe IBA as an auxin either.

The case for 4-Cl-IAA and PAA is more complex; in both cases auxinic activity has been observed, although PAA does not have as strong effects as 4-Cl-IAA (Simon and Petrášek 2011). There is evidence of PAA binding to both ABP1 and canonical auxin signalling proteins, but it inhibits carrier-mediated auxin transport (Simon and Petrášek 2011; Strader and Nemhauser 2013). On the other hand, 4-Cl-IAA has strong auxinic effects, but has also been found to modulate processes that IAA does not (reviewed in Simon and Petrášek 2011). This suggests that PAA and 4-Cl-IAA are related but fundamentally different signals to auxin, which could perhaps be described as "auxin-like" signals. One possibility is that PAA and 4-Cl-IAA might have evolved in certain plant groups by neofunctionalisation from the core auxin synthesis and signalling pathways; certainly 4-Cl-IAA has only been identified in a few taxonomic groups. However, the genetic basis for PAA and 4-Cl-IAA synthesis and signalling is not clear at the moment, and further work will be needed to establish their exact relationship to the primary IAA signal.

Following these arguments to their logical conclusion, one option is to define auxin as IAA and only IAA. Our rapidly developing understanding of auxin perception raises the possibility that more specific assays could help provide a stricter definition of auxin. Excellent work has elucidated the structure of the auxin binding pocket in the TIR1/AFB family of auxin receptors (Tan et al. 2007). Binding of auxin in this pocket promotes association with members of the Aux/IAA transcriptional repressor family, with the auxin acting as a molecular glue between the two proteins. Different Aux/IAA-TIR1/AFB combinations have different affinities for IAA and auxin analogues, leading to the idea that these two protein families act as co-receptors (Calderón Villalobos et al. 2012; see also Chap. 6). Assessment of binding affinities of IBA, 4-Cl-IAA or PAA could allow them to be ruled in or ruled out as auxins. Similar evidence could be derived from studies of ABP1-type receptors, for which the crystal structure predicts different binding affinities for auxin and auxin analogues (Woo et al. 2002).

4 Auxin Is Not a Hormone

Auxin is produced in many tissues and is detected in both the same and other tissues. It is a mobile molecule that can be actively transported over long distances. These properties should be enough to convince anyone that auxin is a signal, but what kind of signal? A word very commonly used in conjunction with auxin is "hormone". In its infancy auxin was often called a "plant growth substance"—*the*

growth substance, even (from the German "Wuchsstof")—but there are also early references to it as a hormone (e.g. Snow 1935), and this latter terminology has been widely used, though by no means universally accepted, for a considerable time now. A general definition of a hormone is a substance secreted by specialised cells in one tissue that affects the behaviour of cells in another tissue in a specific manner. Given what we now know about auxin—it has no specialised site of synthesis or action, nor does it have specific effects on cells—it is clear that, whatever else it might be, it does not meet this definition of a hormone.

It could be argued instead that auxin is a "phytohormone": something quite different to the classical concept of a hormone from animal biology defined above. However, given the disparate substances that are usually classed as phytohormones, and the disparate ways in which they work, how exactly can "phytohormone" as a concept be defined? Any definition that included auxin would have to be so vague as to render the concept meaningless. None of this is to say that the hormone concept cannot be applied to plants. The FLOWERING TIME (FT) protein is a much better fit to the classical hormone concept, but is referred to as hormone only infrequently. Similarly, the CLAVATA3 peptide acts in quasi-hormonal manner, albeit over very small distances. We may also eventually come up with a set of properties that defines a separate "phytohormone" concept that includes the lower molecular weight signals from plants, including for example cytokinins and brassinosteroids. Auxin, however, is really something unique, and even the properties of auxin *itself* are difficult to unify into a single concept.

In some ways, this is a rather semantic argument and a rose by any other name would smell as sweet. However, as described above, it is likely that the tacit acceptance of the hormone concept for auxin creates artificial expectations and limitations in our research. We call auxin a hormone, so we expect, sub-consciously perhaps, that auxin will *act* like a hormone. It is reflected in our experimental designs, in our interpretations of data, and in the way that we write about auxin. To give an example; when TIR1 was identified as a proto-typical auxin receptor, there was widespread surprise that the signal transduction pathway was so short (Kepinski and Leyser 2005; Dharmasiri et al. 2005; Callis 2005; Abel and Theologis 2010). We previously knew little about auxin perception, so why should we have been surprised by this particular answer? We were surprised primarily because we conceptualised auxin as a hormone, and most hormones do have long and complicated transduction pathways, full of exotic sounding kinases and GTP-binding proteins. Yet, we knew at the time that auxin was capable of generating incredibly fast transcriptional responses, indicative of a short signal transduction pathway.

However, the real problem with the hormone paradigm runs much deeper. It is absolutely implicit in the concept that hormones are an *instruction* to the target cells to *do* something in particular (e.g. store glucose! moult! flower!). Similarly, it is implicit in our view of auxin that it is an instructive factor, i.e. that it makes cells *do* things. This leads to the "complexity problem"; if auxin is indeed an instruction, how can it be taken to mean so many different things in so many places? An instruction that can be so liberally interpreted is not instruction at all, and this alone

should convince us that we are looking at the problem from the wrong angle. Whichever way it is approached, the complexity problem certainly needs an explanation, but perhaps if we stop viewing auxin as an instruction, we can make life easier for ourselves and gain a deeper understanding of the auxin enigma.

It would clearly be wishful thinking to hope that the use of "hormone" to describe auxin might be phased out—in the absence of another convenient category, auxin will probably have to live with its label. Still, auxin is not a hormone. It is *far* more interesting than that.

5 Auxin Is Impetus

This list of things that auxin does grows longer by the month. No one working on auxin today needs to be convinced that auxin activity is complex, though some of the pioneers of auxin research might be a little terrified if they had to catch up on the literature. The "complexity problem", outlined above, is the absolute crux of auxin research; how can we understand a signal that has seemingly limitless powers?

One answer to this question is that the canonical auxin signalling system translates auxin into different responses depending on concentration and context (e.g. Kieffer et al. 2010). There is increasing evidence that there are different AFB-Aux/IAA-ARF complexes expressed in each cell type, each with a different affinity for auxin, and different sets of target genes, with the result that in each context a different set of ARFs at a different set of promoters will be activated, with different dynamics (Kieffer et al. 2010; Abel and Theologis 2010). In *Arabidopsis*, there are 6 AFBs, 29 Aux/IAAs and 23 ARFs and therefore a very great number of possible combinations that could operate in different cell types at different times to provide specificity in auxin response (e.g. Rademacher et al. 2011). It is certainly clear that these different complexes have highly quantitative effects on auxin signalling due to differences in the stability of the Aux/IAAs and differences in the affinity of the components for each other (Havens et al. 2012). Since they are the DNA-binding components of the system, the main qualitative effects of auxin signalling on transcriptional output (i.e. which set of genes is activated) are probably mediated by the combination of ARFs that are present in each cell. However, recent work suggests that there are only five major auxin-activated ARFs in *Arabidopsis* (ARFs 5, 6, 7, 8 and 19), meaning that the multiple effects of auxin cannot be explained simply as a function of which ARFs are activated. The remaining ARFs (the so-called repressive type) only interact weakly with the core auxin signalling machinery (Vernoux et al. 2011) and are therefore probably not activated by auxin in themselves; instead, it seems likely that these proteins are cell-type specific inhibitors of auxin response. If these ARFs block specific promoters, they could determine the subset of genes available for activation by positive ARFs in given cell types, thus contributing to the specificity of auxin response; however, there is currently little evidence that repressive ARFs do act in this manner. Indeed, recent work shows that ARFs probably have few intrinsic differences in their

DNA-binding specificity, although it was demonstrated that ARFs act as dimers and that ARF1 and ARF5 dimers have different tolerances for the spacing between pairs of auxin response elements (Boer et al. 2014), so some specificity may arise from the configuration of promoter elements in target genes. The overall impression is that we cannot answer the complexity problem through canonical signalling alone. Where does this leave us? There are of course other signalling systems for auxin; indeed, given the ubiquity of auxin and its very long evolutionary history, it would be rather surprising if there were not. In addition to ABP1, there are tentative suggestions in the community about other possible receptors or signalling components. We should certainly expect more receptors to emerge in the forthcoming years.

As presaged above though, the simplest solution to the complexity problem may be that auxin is not actually an *instruction* to do anything at all; in which case, the concern about how auxin causes so many responses could be reframed and perhaps better understood. However, if auxin is not an instruction, then what kind of signal is it? A previous review by Stewart and Nemhauser (2010) proposed that auxin may act as a kind of cellular currency, permitting many different "transactions" to occur, the nature of the transaction depending on exactly where the auxin is being "spent"; auxin here is seen as a "permissive" type of signal.

The key element of this approach is the switch in focus from an auxin-centric viewpoint to a process-dominated one. Perhaps auxin can be best understood if it is viewed as a signal that provides "impetus" to processes, but does not specify what those processes are. Thus, auxin might actually *do nothing*, but rather motivate everything. In this model, each cell type has a set of processes that are inherent to it (i.e. are developmentally specified), any of which might be "boosted" by auxin, but none of which is directly specified by auxin. To put this in a molecular framework, whether an auxin-influenced gene is "on" or "off" depends on the *other*, cell-type specific transcription factors bound to the promoter of that gene, while the ARFs binding to the promoter act as a kind of rheostat, specifying how much transcription occurs—but ultimately do not control specificity. To give an example, we might imagine a cell type (A) that can differentiate into a second cell type (B) under the influence of a gene (C). C is normally expressed in A, but not at high enough levels to trigger differentiation; however, when auxin is present, transcription of C is boosted, leading to differentiation into B. Thus, auxin does not specify the differentiation of B—that is implicit in the developmental context of A—but it would *appear* to the observer that auxin is the causative factor for B. Auxin does not cause differentiation to B in other contexts, because C is only ever expressed in the context of A; auxin specifies neither C activity nor B formation and can only drive those processes in the context of A.

While this might not seem very different from current models of auxin action, in many ways that is the whole point: it is a subtle shift in emphasis, but it shifts the complexity problem away from auxin. Under this lens, auxin action is not actually complex; the complexity is in the tissue systems themselves, and the ways in which they each utilise auxin as an impetus to drive different processes. The recent theoretical struggle with auxin has been to try and derive 100 qualitatively different

instructions from one molecule; the resolution to this problem may simply be that there is only one auxin signal—impetus.

6 Auxin Is Complex

Pleasingly simple though this concept is, auxin will never be *that* straightforward. This "impetus" model may particularly useful in developmental responses to auxin (as contrasted to "simple" growth responses), especially the more subtle patterning effects such as gynoecium development, embryo patterning, etc. The impetus model may also explain the long-standing puzzle of the role of auxin in cell division. Auxin is often seen to regulate cell division (particularly in callus, for instance), and it has long been suggested that it may be a general regulator of division, directly integrated with the cell cycle machinery (e.g. den Boer and Murray 2000). While there is now some evidence for a direct cell cycle effects (Jurado et al. 2010; see also Chap. 7), it is also clear that auxin does not universally promote division. However, if the general role of auxin is to potentiate processes that are already specified, then its role in cell division would become clearer; auxin promotes division (by canonical signalling and/or other pathways) only in contexts where the potential for division is already developmentally specified; for instance, the root meristem (Sabatini et al. 1999), or cambial cells (Snow 1935).

The impetus model will not explain all aspects of auxin action. For instance, there could be some developmental processes in which auxin does act as an instruction, specifying events rather than just driving them; the specification of vasculature, which can often happen out-of-context (Sachs 1981; Sauer et al. 2006), might be an example. Moreover, the difference between instruction and impetus might be blurred in some developmental processes. Auxin has long been considered a general regulator of cell elongation—indeed, this is its proto-typical function, for which it is named—and the many "classical" growth responses to auxin are manifestations of this same phenomenon. The prominence of this response makes it tempting to conclude that a principal, and perhaps ancestral, role of auxin is as an instruction to cells to elongate, but it is nevertheless also clear that elongation is highly context dependent.

Thus, even if we can understand how complexity emerges in responses to auxin mediated by canonical signalling, it may not be possible to explain all these transcriptional effects in a single convenient concept. Add to this the poorly understood role of ABP1-dependent signalling, and the probability of other minor signalling mechanisms, and it is clear that complexity is not merely an artefact of "observer effect", but a real feature of auxin responses—at least in the flowering plants that are most commonly studied. Complexity does not lie just with signalling systems however, because when auxin transport is factored into the equation, things become even more convoluted.

7 Auxin Is Connectivity

It is not generally remarked upon, but the properties of the auxin transport system (see also Chap. 5) are not particularly well suited to the actual transport of auxin. Admittedly, it is better than diffusion, but transport rates in the order of 1 cm/h are scarcely impressive, and repeatedly transporting auxin into and out of cells is very inefficient. As an engineering solution, transporting auxin in the phloem or xylem (as does happen to some degree) would be much more effective for either signal transmission or bulk movement of auxin as a "commodity". We might therefore conclude that auxin transport is not really about transport per se, but rather *connectivity*. What the transport system very effectively achieves is to connect every cell in the plant together, in an intricate web of auxin, flowing slowly but inexorably from shoot to root. An important implication of the "impetus model" is that auxin has a simple, quantitative informational content, interpreted by cells to modulate cellular processes. Viewed in this perspective, the transport system can therefore be seen a system for distributing this information to cells. However, it is much more than that; it is not simply a one-dimensional flow of information; the auxin stream is constantly altered as it moves through cells and tissues, and further spatial and temporal stimuli can be integrated into the system en route. In this model, moving auxin through cells (rather than bypassing them) is a key feature of the system, because it allows all cells both to connect to the system and to modify it. Other important properties of the system in this respect are that it is hierarchical—so that, for instance, some cells/tissues have disproportionate input into the system or disproportionate exposure to the transport stream—and it is directional—so that the propagation of information is not equal between all cells or in all directions.

All cells can modify, in reflection of their own status, the amount of signal they pass on to neighbouring cells by adding or subtracting auxin, or by changing the immediate kinetics of transport. Due to the high connectivity of the system, these modulations can have both local and global effects, meaning cells can influence multiple processes on a global scale, without having to emit multiple signals. Of course, the effect of a single cell will be minimal, but coordinated action by a group of cells (a meristem, for instance) would be able to alter significantly development across the whole plant. Overall, the topology of the auxin transport system permits auxin to have a much greater influence over development than if it were merely a long-distance signal, travelling through the vascular tissues. This is not to argue that this topology is inherently advantageous per se—indeed, for other signals which mediate specific effects, direct transmission is much preferable—only that in the peculiar case of auxin this highly connective method of transport greatly increases the influence and complexity of this singular signal.

As an example, we can consider the *Arabidopsis* root meristem. At the hub of this system is the tip of the root; the quiescent centre (QC), which acts as the organising centre of the meristem, and the columella root cap, which is particularly important in detecting environmental stimuli (including gravity) and directing root

growth accordingly. Auxin promotes cell division and elongation in the root meristem and also drives tropic behaviour in the growth of the root (see also Chap. 16). These effects are mediated by basipetal/shootward streams of auxin moving from the root tip through cell files in the lateral root cap and epidermal layers of the root. The columella controls allocation of auxin into these cell files, and by varying the distribution of auxin between two sides of the root can control asymmetric root growth. Auxin can also be produced in the root tip, allowing the root to control the amount of growth (as distinct from the direction of growth) in relation to local stimuli (Stepanova et al. 2008). Here, auxin is being used as an informational signal to connect the "sensory" cells in the root tip, which control the amount and distribution of auxin, to the meristematic cells where auxin provides impetus for cell division. In theory, the meristematic cells could synthesise their own auxin to drive division, but the lack of connectivity with the tip (and by extension with other meristematic cells) would prevent whole-organ spatio-temporal coordination of growth; so in this context, the key feature of auxin transport is the connectivity it allows, and not the final distribution of auxin that it produces. Furthermore, as is long established, the roots act as a sink for shoot auxin, and there is certainly bulk transport of auxin from shoot to root (e.g. Bhalerao et al. 2002). There is thus a rootward stream of auxin, linking active shoot meristems with active root meristems, that developmentally connects the shoot and root. This auxin stream is not required to drive root growth, on short timescales at least, since the roots can both synthesise their own auxin and recycle the existing pool (Stepanova et al. 2008; Grieneisen et al. 2007), but it allows global root growth to be modulated with respect to shoot growth. Again, connectivity is the key feature of the system and not the supply of auxin per se. The auxin from the shoot moves through the centrak cell files of the root, to the tip, where this global signal can be integrated with the local auxin stimuli. The root tip cells can either add auxin to the pool, or remove it, and spatially distribute the auxin as appropriate, to generate an integrated impetus signal tailored to the circumstances of that individual root meristem (see also Bennett and Scheres 2010). The directionality of the system means that roots cannot back-signal to shoots directly through transport of auxin; instead, roots produce other signals, such as strigolactones, which are transported in the xylem (Kohlen et al. 2011) and act to regulate PIN1 protein abundance in the shoot (Crawford et al. 2010; Shinohara et al. 2013), thus allowing shoot growth to be regulated with respect to root growth and establishing feedback between the two tissue systems.

We can thus observe that the connectivity of the auxin transport system allows many different stimuli, local and global, to be integrated into a single quantitative signal at the point of effect. The auxin transport system greatly increases the apparent, and indeed actual, complexity of auxin responses, since to understand how auxin "does" so many things, it is not merely enough to know how much auxin is in a given tissue and how that tissue will respond to the auxin; it is also important to know how the auxin is transported through the tissue, and how the tissue modifies the signal. However, a further dimension of complexity still is added by the remarkable emergent properties of the auxin transport system.

8 Auxin Is Spooky

When it comes to astonishing insights into the very nature of the universe, Albert Einstein certainly has a good publication record, including many contributions to the development of quantum mechanical theory. One of the emergent properties of this theory is "quantum entanglement", which suggests that particles that physically interact and are then separated continue to be "entangled"; they instantaneously detect and respond to changes in each other's state, violating the speed of light in the apparent transmission of information between them. This was a step too far for even Einstein, who dismissed the notion as "*spukhafte Fernwirkung*" ("spooky action at a distance"), although entanglement has since found considerable experimental support and is now a well-established part of standard models of quantum theory. Einstein made rather fewer memorable contributions to the field of auxin research—one cannot help but feel that he ducked the *really* difficult questions—but perhaps he was put off by plant science's own version of the entanglement problem and the rather spooky effects generated by the auxin transport system.

It is generally accepted that auxin transport streams are self-organising, although we still have little grasp on how such self-organisation occurs (see also Chap. 14). Many models have been proposed, and mathematically analysed, to explain the emergence of various types of auxin transport phenomena (particularly "up-the-gradient" and "with-the-flux" patterns), but those models do not capture all phenomena and also lack an explicit mechanistic basis for core aspects of their operation. It is currently a key challenge in auxin biology to unite the experimental and theoretical aspects of this problem into a cogent explanation of these self-organising behaviours. From the point of view of the "auxin question", the most interesting aspects of self-organisation in the auxin transport system are those properties that apparently allow auxin to act as a "signal" between cells or tissues without any actual movement of signal from one to the other. In essence, it seems that because cells and tissues are "entangled" through the transport system, events in one location can affect auxin transport in other (non-downstream) locations, allowing cells to detect those events and respond accordingly to them, without any actual movement of auxin (or other signals) between the two locations. This is, as Einstein obviously feared, the botanical equivalent of action at a distance; and as far as attempting to explain auxin action to a 6-year-old goes, it is probably the final nail in the coffin. It is not enough to know where auxin is, or where it is going; the connectivity of the whole system must also be understood.

The starkest example of this action-at-a-distance is seen in Sachs' classic canalisation experiments (Sachs 1981). For instance, an auxin source (A), connected to a vascular bundle with high levels of auxin transport (V), will prevent a second auxin source (B) from establishing a transport connection with V, but if A is removed then a connection between B and V is established. Both the "finding" of V by B and the inhibition of that process by A occur without any apparent signal transmission between V and B, or A and B, but as somewhat *spooky* properties of the system as a whole. Further examples of these emergent properties can be seen in

the regulation of vein initiation in leaves (Sachs 1969; Rolland-Lagan and Prusinkiewicz 2005; Scarpella et al. 2006), phyllotaxis in the meristem (Smith et al. 2006; Jönsson et al. 2006; see also Chaps. 10 and 15) and in the regulation of axillary buds by auxin exported from active meristems (Prusinkiewicz et al. 2009).

9 Auxin Is Ancient

In the preceding sections, we have tried to provide a framework in which the intricate complexity of responses to auxin can at least be contemplated, but there is no escaping that complexity, and no set of simple rules that can predict what those responses will be. Ultimately auxin can only be understood as a function of the whole system, and reductionist approaches seeking to explain auxin responses in terms of simple molecular events will be limited in their power to do so. To the parsimonious mind, this naturally poses the question of *why* the system is so complicated—how and why did the architecture of the system evolve in this way and not as a system of many separate signals? It is possible that by studying the evolution of auxin, we can begin to unravel some of that complexity and ask whether auxin was ever simple, and if so, what its proto-typical functions were. To misquote Dobzhansky: "nothing to do with auxin makes sense except in the light of evolution".

At least part of the problem in understanding auxin is that there are no real precedents on which to base an explanation; such a generalist signal does not, to our knowledge, exist in other systems and even in plants, auxin is a unique molecule. We must therefore try and understand which particular aspects of plant biology might have promoted the evolution of a signal with these properties that are not present in other systems; the quest to understand auxin thus draws us into questioning the very nature of plants themselves. Since there is convincing evidence for auxin synthesis, response and transport in streptophyte algae, the sister taxa to land plants—indeed, it seems that all the main elements of auxin biology were in place before the evolution of land plants (De Smet et al. 2011; see also Chap. 13)—the answers must at least partially lie underwater and in the past.

Photosynthesis is undoubtedly the quintessence of plants, and at heart a plant is a machine for optimal extraction of light and other key nutrients from the environment. We should therefore expect that early in algal/plant evolution the major factors driving (and constraining) development were environmental cues. A second defining characteristic of plants is the cellulosic cell wall, which also plays a key role in determining the way plants develop. While the cell wall absolutely does not preclude complex cell shapes/differentiation pathways, it does promote the use of relatively undifferentiated cells with simple morphology in development unless there is very good reason to use something more complex. Development in plants therefore tends to be more a consequence of the ways cells are arranged at a tissue level, rather than due to major morphological changes in the cells themselves

(though of course there are counter-examples). The plant cell wall also allows two fundamentally different mechanisms of "growth" to occur: cell division, preceded by cell growth in an energy-intensive process, and cell expansion, driven primarily by water-driven expansion of the vacuole, which is in turn made possible by the rigidity of the cell wall. Together, photosynthesis and the cell wall seem like good candidates for plant-specific processes that might have promoted the evolution of auxin, especially in the algal context.

Although many green algae do have complex morphology and differentiation, these are clearly derived forms, and most green algae assume simple morphologies such as filaments or laminae. We can use Coleochaete as an example; these freshwater algae have simple development, producing planar discs of tissue or branching filamentous structures that expand in size with relatively little further differentiation. Like all plants, the ultimate selective force underlying development in Coleochaete must be to optimise resource acquisition to support reproductive effort, and an important determinant of the development of Coleochaete is therefore light harvesting capacity. We would expect the main drivers for growth in these organisms to be the availability of light, together with CO_2, nitrogen, phosphorous and other minerals. The inputs into growth are therefore quite complex, but the low developmental potential of the system means that the output is very simple; in discoidal Coleochaete, the disc must expand in a more or less even-fashion, through a combination of cell expansion/elongation and division. Indeed, the cell divisions in this system follow as a natural consequence of cell expansion, and their orientation is apparently determined by the dimensions of the cell (Dupuy et al. 2010), so the primary developmental output is actually coordinated cell expansion. From a parsimony perspective, it would make some sense to integrate the various inputs into a single signal that controls this primary developmental output; and of course, that signal would closely match the properties of auxin. It is currently unknown whether development in Coleochaete is controlled in this manner, or whether auxin plays any role, but given the central role of auxin in cell expansion in land plants, it seems like a reasonable hypothesis.

Regardless of how development is actually regulated in Coleochaete, we can at least rationalise the evolution of an auxin-like signal in an organism that is environmentally sensitive and has low developmental potential. This hypothetical auxin-like signal would also have two further important properties. Firstly, it is a widely distributed signal containing integrated information regarding the environment that could be harnessed to regulate any other process that also required environmental input. Secondly, although it is acting as an "instruction" for growth, the signal is highly generic in its effect, and is not tied to any particular developmental or differentiation pathway; it affects primarily growth rather than development. Thus, utilising this signal for other purposes does not mean that those processes would only happen in a certain context; the signal is contextually neutral. We could therefore imagine that for a new developmental process that was coordinated with the environment—the production of rhizoid-like cells for instance—our hypothetical signal could be used to give "impetus" to the process, but would not directly specify the differentiation of these cells. This would not interfere with

the expansion-promoting role of the signal, nor would the expansion role interfere with the production of rhizoids. Any number of processes can subsequently "piggyback" onto the signal in the same way, because the signal is still not tied to any particular developmental context. If the hypothetical signal had a simple transcriptional output (like auxin), then piggybacking onto the signal could simply be a case of introducing appropriate binding sites into the promoters of relevant differentiation genes—a very simple process that can evolve quickly by single base-pair modifications.

We can thus see how an auxin-like system might suit simple algal developmental systems. We can also see that, if such a simple generic signal evolved, it would subsequently provide a very cost-effective way of providing the same information to other processes—because it encodes powerful informational content, and yet has little instructional value. Attuning many processes to the same signal would also allow the efficient co-regulation of many processes in line with the general status of the plant. It is clear that in *Arabidopsis*, a huge proportion of the genome is regulated by auxin, which makes little sense as a response to a specific hormonal signal, but much sense in the context of a universal co-regulator. The power of such a system lies in the initial integration a range of environmental (and perhaps other) inputs into a single signal that controls growth in a generic way; once such a signal existed, it would perhaps be inevitable that this rich vein of information would be tapped over and over again. Of course, there is currently little evidence to suggest that auxin did initially evolve in such a manner, but it is at least a plausible and testable hypothesis. Ultimately, it is clear that auxin exists in and may well regulate growth in green algae; and therefore that as far as understanding the origins of complexity goes, a key future direction for auxin research lies in the past.

In higher plants the homeostasis, transport and signalling of auxin, coupled with the extensive feedback regulation of all these processes by auxin itself, adds up to a staggeringly complex network, usually characterised in terms of loops and more loops (Benjamins and Scheres 2008; Leyser 2010). However, auxin must have started as a simple algal signal, and even alongside its complexity, auxin in modern plants still possesses the kind of simple, generic and universal characteristics that we have discussed in this section. Although it has existed for a very long time, auxin has not undergone the type of sub- or neofunctionalisation that typically occurs in peptide signals (for instance, insulin-like growth factors from animals, or CLE peptides from plants) that would have allowed repeated separation of accumulated functions into new signals, and the subsequent streamlining of each new signal. We must therefore assume that the value of auxin as a universal signal has been sufficiently high throughout plant evolution to retain it as a single signal and to warrant the evolution of this tangled network of regulatory proteins, which allow the information encoded in the signal to be deciphered and utilised in an ever-increasing number of ways. Thus, the ultimate paradox of auxin might be that its complexity is simply an emergent property of its elegant simplicity.

10 Conclusion

What is auxin? It is a question that operates on several philosophical levels, from the categorical—*of what type is it?*—through the metaphysical—*what is its essence?*—to the logical—*how does it work?* The urge to categorise is one of philosophy's oldest motivations, but auxin is difficult to classify in a meaningful biological way. As far as we can tell, it is a unique kind of signal, both in its myriad functions and in the complexity of responses to it; to try and put a label on auxin is to obscure its singular nature. Thus, at the simplest level, the somewhat unsatisfactory answer to the auxin question is that auxin is *auxin*, no more and no less.

However, another major motivation of philosophy is to explain the enigmatic—auxin certainly qualifies in that respect—and it is possible to give rather more satisfactory answers to the deeper aspects of the auxin question. In this chapter, we have attempted both to explore the meaning of the question and to formulate some answers to it: not definitive ones, and indeed mostly speculative ones, but hopefully at least inspiring and testable ones. Ultimately, the auxin question is answerable—although definitely serpentine, it is not venomous—but the answer is still very long. It is already as long as this book, and it is likely to continue growing for some time; after all, auxin has been growing plants for perhaps a billion years, and we have only barely just noticed.

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Chapter 2
Auxin Biosynthesis and Catabolism

Yangbin Gao and Yunde Zhao

Abstract Auxin concentrations in plants are tightly regulated through both biosynthesis and degradation. In the past few years, much progress was made in the area of auxin metabolism. Genetic and biochemical studies in *Arabidopsis* unequivocally established a complete tryptophan (Trp)-dependent two-step auxin biosynthesis pathway in which Trp is first converted into indole-3-pyruvate (IPA) by the TAA family of aminotransferases and subsequently indole-3-acetic acid (IAA) is produced from IPA by the YUC family of flavin monooxygenases. The TAA/YUC pathway is highly conserved in the plant kingdom and is probably the main auxin biosynthesis pathway in plants. Recent work also demonstrated that oxidative degradation of auxin plays an essential role in maintaining auxin homeostasis and in regulating plant development. In this chapter, we discuss the recent advancements in auxin biosynthesis and catabolism.

1 Introduction

Auxin is an essential hormone for many aspects of plant growth and development (Zhao 2010). Plants have evolved a sophisticated network to control auxin levels with spatial and temporal precision in response to environmental cues and developmental signals. Indole-3-acetic acid (IAA), the main natural auxin in plants, can be produced from de novo biosynthesis. Free IAA, which is the presumed active form of auxin, can also be released from IAA conjugates including IAA esters, IAA-saccharides, and IAA-amino acids. A third probable route for producing IAA is to convert indole-3-butyric acid (IBA) to IAA using enzymes similar to those

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E. Zažímalová et al. (eds.), *Auxin and Its Role in Plant Development*,
DOI 10.1007/978-3-7091-1526-8_2, © Springer-Verlag Wien 2014

used in β-oxidation of fatty acids. When auxin levels need to be lowered, plants employ several mechanisms to deactivate IAA. IAA can be quickly converted into the presumed inactive forms by reaction of the carboxyl group of IAA with amino acids, sugars, and other small molecules. The IAA conjugates may serve as a first step for the eventual complete degradation of IAA. IAA is also inactivated by oxidation of the indole ring of IAA. For example, IAA can be converted to 2-oxindole-3-acetic acid (OxIAA). In this chapter, we discuss the progress made in the area of auxin biosynthesis and metabolism in the past few years.

2 De Novo Auxin Biosynthesis

De novo auxin biosynthesis is broadly divided into two categories: Tryptophan (Trp) dependent and Trp independent. Trp-independent auxin biosynthesis pathway was proposed two decades ago based on results from feeding plants with labeled Trp and Trp biosynthetic intermediates and from studies on Trp-deficient mutants (Wright et al. 1991; Normanly et al. 1993). However, the molecular mechanisms and genes for the Trp-independent pathway are not known. Therefore, the Trp-independent pathway will not be discussed further in this chapter.

Trp has long been known as a precursor for the production of IAA in plants. Feeding plants with labeled Trp yields labeled IAA, indicating that plants have the enzymes to convert Trp to IAA (Wright et al. 1991; Normanly et al. 1993). Many biosynthetic pathways have been elucidated using analytic biochemistry techniques in combination with labeled precursors and intermediates. For example, the biosynthetic routes for brassinolide and ethylene have been established long before the biosynthetic genes have been identified (Yang and Hoffmann 1984; Sakurai and Fujioka 1993). However, the classic feeding and analytic biochemical approaches failed to identify the key components for Trp-dependent plant auxin biosynthesis pathways. There are several reasons for this apparent failure. First, Trp is a precursor for many metabolites (Fig. 2.1). Trp is a precursor for indole-3-pyruvate (IPA), tryptamine (TAM), indole-3-acetaldoxime (IAOx), indole-3-acetamide (IAM), indole-3-acetonitrile (IAN), and indole-3-acetaldehyde (IAAld) (Fig. 2.1). *Arabidopsis* and many other plants have the capacity to produce all of the above-mentioned intermediates (Fig. 2.1) at a given developmental stage (Ouyang et al. 1999; Sugawara et al. 2009). Some of the intermediates such as IAN exist in very high concentrations (Fig. 2.1) (Sugawara et al. 2009). Such a complex profile of Trp metabolism makes it difficult to identify Trp-dependent IAA synthesis intermediates. Second, some of the intermediates are intrinsically unstable in vitro and can be nonenzymatically converted to other compounds during the experimental process, therefore complicating the analysis of metabolic profiling. For example, IPA is readily converted nonenzymatically into IAA in vitro (Bentley et al. 1956). Third, most of the Trp metabolic intermediates display auxin activities during in vitro bioassays (Fig. 2.2). In the presence of IAM in growth media, light-grown *Arabidopsis* seedlings have long hypocotyls and epinastic cotyledons

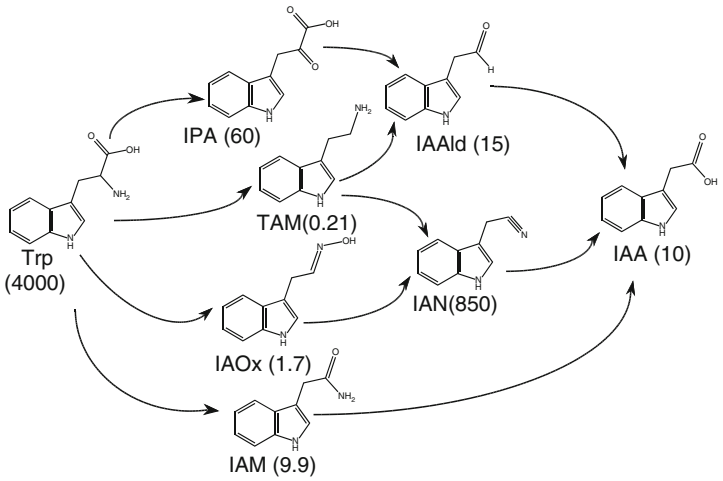

Fig. 2.1 Selected tryptophan metabolic intermediates. *Arabidopsis* plants produce all of the intermediates shown in the figure. The numbers in parenthesis refer to the actual concentrations in ng/g fresh weight. *IAA* indole-3-acetic acid, *IAAld* indole-3-acetaldehyde, *IAOx* indole-3-acetaldoxime, *IAM* indole-3-acetamide, *IAN* indole-3-acetonitrile, *IPA* indole-3-pyruvate, *TAM* tryptamine, and *Trp* tryptophan

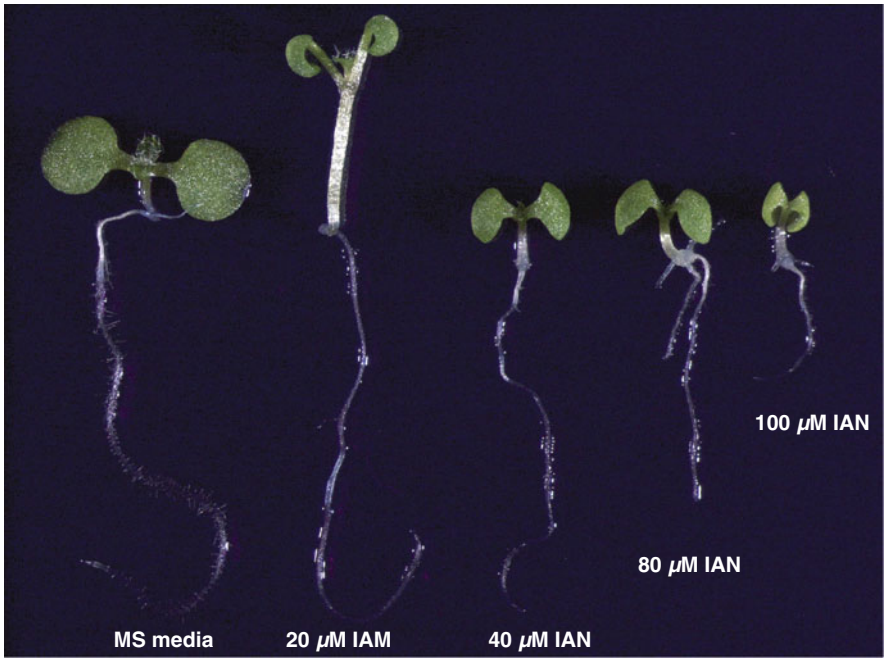

Fig. 2.2 Some tryptophan metabolites display auxin activities. Indole-3-acetamide (IAM) stimulates hypocotyl elongation and causes epinastic cotyledons. Indole-3-acetonitrile (IAN) inhibits primary root elongation and stimulates adventitious root initiation