

## 1

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# Introduction

Although it was evident from Darwin's studies of tropisms in plants that informational signals passed from one part of the plant to another, the proof that it was a chemical substance that passed awaited the famous *Avena* coleoptile experiments of Frits Went (1928). These showed that a molecule (later identified as indole-3-acetic acid [IAA]) was the active agent that was water soluble and would pass across an agar barrier placed between one tissue and another – in his earliest experiments this was between the coleoptile tip (producing IAA) and the IAA-regulated elongating region of the coleoptile below. A tremendous amount of work, both in studying the physiology of this response to IAA, and in identifying the many analogues to IAA, sought the molecular structures required to provide an active molecule. It was from this highly intensive period of plant physiology study that the agricultural revolution of herbicides, defoliant and growth regulators of the 1940s and 1950s was originally generated.

But it was the insect physiologists with their identities of hormone-producing glands and hormone-responding tissues remote from the glands who developed the concepts of target tissues, signalling molecules and receptor sites. Perhaps the most spectacular to record, as an example of the approaches followed later by plant scientists, is the work in the 1930s and 1940s concerning the processes of moulting of larval epidermal skins and of metamorphoses to the adult state (Karlson, 1956).

The prothoracic gland produces the steroid hormone ecdysone (the moulting or juvenile hormone) that is transported in the haemolymph to the insect epidermis. Along the way, ecdysone binds to specific proteins and then at the site of moult, induces enhanced transcription and modification of coordinated gene activity. Importantly, ecdysone-induced puffing at specific sites in polytene chromosomes could be demonstrated in salivary glands and related to each developmental stage of differentiation. The insect field was set for determining the

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## 2 HORMONES, SIGNALS AND TARGET CELLS

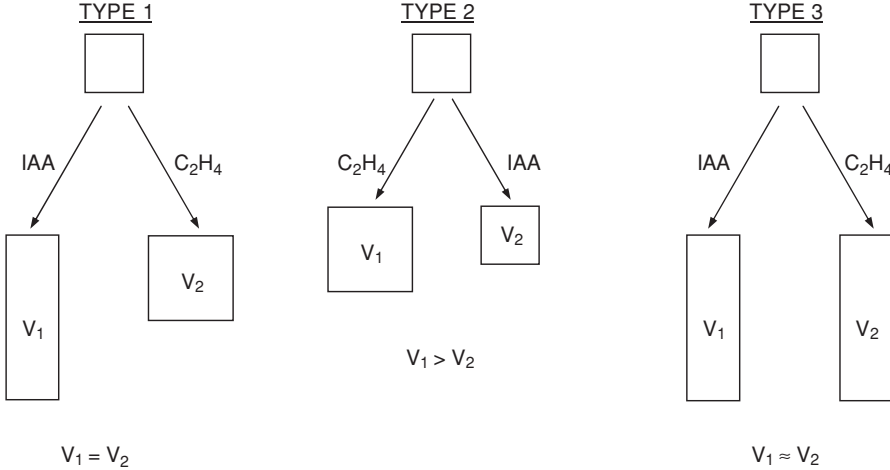
cascade events of signals, receptors and the hormonal regulation of gene expression in specific target cells. The plant field followed fast; but in the absence of specific glands or polytene chromosomes and the absence of distinguishably specific target cells the now recognised parallels between plant and animal signalling were more difficult to explore and took longer to resolve.

The concept of target cells in plants arose originally from the knowledge that cells of coleoptiles and etiolated shoots would enlarge and extend in response to auxin and that ethylene would arrest such elongation growth. Those of us who worked in the field of abscission became equally aware that the cells that made up an abscission zone enlarged prior to their separation and did so with alacrity if exposed to ethylene, but if given auxin these same cells would neither grow nor separate. The insect developmental biologists were aware that only certain cells would perceive and respond to particular hormonal signals in a particular way. It became inevitable to learn that although most plant cells might look the same, they were all as individual and distinct from one another as those making up the highly responsive organs of mammals and insects. As the cuticular cells of a larva were targets for the moulting hormone ecdysone, and the mammalian liver for insulin, so the cortical cells of the plant shoot were targets for auxin and ethylene. The concept for target cells in the developmental biology of plants was born.

A first substantive evidence for different target types in cortical cells arose from observations of the differences in their growth responses to auxin and ethylene. Whereas the immature cells of young dicotyledonous shoots such as those of the pea *Pisum sativum* will elongate in the presence of the auxin passing downwards from the meristem, the addition of ethylene to those shoots will cause them to arrest elongation growth and instead to expand laterally, with the cell volume remaining essentially unchanged and determined by the availability of auxin from the meristem (Osborne, 1976). Cells of abscission zones, however, such as those in the leaves of the bean *Phaseolus vulgaris*, behave in a quite different way: their expansion is enhanced by ethylene but not by auxin. The existence of a third type of cortical cell is found in the stems and petioles of many species of flooded or aquatic habitats. *Ranunculus sceleratus* or the water fern *Regnellidium diphyllum*, for example, possesses cortical cells that will expand and extend with either auxin or ethylene (Figure 1.1). In 1976, these three distinguishable cell types were designated as Type 1, Type 2 and Type 3 (Osborne, 1976, 1977a, b) with respect to their responses to auxin and ethylene.

Once we understood that cells that looked similar to the eye had quite specific responses to hormonal signals, it became evident that strict regulatory controls operated to maintain these coordinated patterns in the cell society. Clearly, not every cell differentiated along the same developmental pathway or had the competence to respond in a similar way to the same hormonal signals. More examples of target cells other than those with a highly specific perception and response to auxin and ethylene signals have now engaged the physiologists and molecular biologists, none less so than the terminally differentiated cells of the aleurone tissue in graminaceous seeds. In aleurone cells, the competence to respond to a gibberellin/abscisic acid control determined their final response and cell fate.

## INTRODUCTION 3



**Figure 1.1.** Overview of Type 1, Type 2 and Type 3 target cells in terms of their responses *in planta* to auxin and ethylene. V = volume expansion

The terms target cell, signal, cellular competence and tissue sensitivity are now used widely when describing the development of eukaryotes, but differences have emerged in terms of the definitive definitions of their meaning. Thus before embarking on our dissection and discussion of the target cell concept in higher plants, it is pertinent to begin with a series of definitions that are used in this volume.

### What is a target cell?

The term target cell was first conceived in animal biology and is generally taken to mean a cell that has a pre-determined competence to respond in a defined way to a specific hormonal signal. Only the specific hormone (or a chemically related analogue) can evoke that particular response in the cell. In plant biology, the definition is essentially the same. For the purposes of this book, we take the view that every plant cell is a target cell for one or more of the plant hormones or other regulatory signals.

### Cells, simple and complex tissues

The body of higher plants arises from the mitotic activity of apical meristematic regions, and thence through cell elongation and differentiation the primary plant body is formed. In the gymnosperms and dicotyledonous angiosperms, and certain monocotyledonous species, secondary growth occurs through additional cells arising from meristems remote from the apex, principally those of the vascular and cork cambium tissues.

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Excerpt

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#### 4 HORMONES, SIGNALS AND TARGET CELLS

The higher plant body is considered commonly to comprise three systems of tissues: the dermal, the vascular and the ground tissues (Esau, 1965). The primary dermal tissue is the epidermis, with periderm tissue forming in secondary growth. The phloem and xylem are the principal vascular tissues, and the ground tissue systems comprise all the remaining tissues, with parenchyma the primary cell type.

Within these tissues, a further layer of complexity emerges with the organisation of cell types. In some tissues, only a single cell type is found and these are referred to as simple tissues. In others, for example the stele, many cell types comprise the tissue, including those that are dead, and these are commonly referred to as complex tissues.

##### How are signals defined?

Within the plant body, cells must, perforce, communicate by chemical or physical means. These intertissue messages are the chemical signals that evoke specific biochemical and molecular events in each responsive target cell. From the first of these signalling molecules to be discovered, namely auxin in 1928, a wide spectrum of such molecules has now been identified and while the chemistry of these compounds is quite diverse they all exhibit certain shared characteristics. Although there are major sites of synthesis within the plant, signals all move readily between cells (either long or short distances) and evoke responses both at the site of synthesis or at sites that are remote. Classically, for a cell to recognise a signal, it must have a receptor for that signal. All cells must, therefore, possess an array of receptor systems, but so far comparatively few of these have been precisely characterised. However, the identification and characterisation of these receptors is currently a very active research area in plant biology and as such represents areas of high relevance to the target cell concept. Accordingly, full chapters are devoted to the identification and characterisation of receptors and their significance in the mechanisms for the relief of gene repression.

The examples of intertissue signal molecules considered in this volume include both old and new. The five major classes of plant hormones (auxin, ethylene, abscisic acid, gibberellin, cytokinins) are well established, but many more endogenously produced molecules with equally diverse structures are now known to induce a range of specific responses in specific tissues. These include

- steroid-like molecules, e.g., the brassinolides,
- low molecular weight compounds such as jasmonic acid and salicylic acid,
- oligosaccharins, including free *N*-glycans, and
- peptides, such as systemin.

It is not our intention in this volume to devote space to considering what constitutes a plant hormone and whether, for example, brassinolide should be included in that group. Rather, our focus is to consider the role of such molecules as signals that convey information to cells in tissues and organs – that is, to the cells that are their recipient targets.

### *Intracellular signal transduction*

Although the question of the mechanisms by which a cell may perceive an external intertissue signal from which it can then interpret a directive in biochemical terms is still open to debate, it is not the central focus of this volume. However, the considerable progress in defining how plant hormone and other signal receptors are evaluated, particularly with respect to the degree to which such characterisation further defines the target cell concept, means that discussion is inevitably included. Signal transduction in the cytosol and nuclei will not be described here in detail, but the authors are aware that the more recent dissection of such biochemical events is of direct relevance to the target cell response. The emerging ideas of hormonal cross-talk are constantly reiterated as each new signal transduction pathway is elucidated. The aim of this volume, therefore, is to interpret such descriptions within the framework of the target cell concept.

### **Cellular competence**

We define cellular competence in both qualitative and quantitative terms. A cell must possess the ability to perceive, transduce and respond to a signal, otherwise it is not a target for that particular input. In plants, unlike animals, all the living cells are exposed to the same hormones, though the signal transduction pathway is not necessarily the same for each target cell. Whereas a positive response to the signal is evidence of a cell's specific target state, lack of response cannot be taken as evidence of a non-target condition. For in every signal input there must be a threshold level that a cell can interpret; this must exceed the presence of non-specific inhibitors and homeostatic controls exerted by specific repressor controls of the target cell response (see Chapter 5). Furthermore, a cell may perceive, transduce and respond to a particular signal in a different way from its neighbouring cell. This we take as clear evidence of their individual and different target states.

### **Cell and tissue 'sensitivity' to signal inputs**

For the purposes of this volume, we define cell and tissue sensitivity in terms of the concentration of inputs necessary to evoke a measurable response. Thus a highly sensitive target tissue requires a low concentration of signal input to evoke a pre-determined response – i.e., display its tissue competence. It should be noted that any one cell may be a target for more than one signal; therefore the sensitivity of the tissue for each signal may vary, and must be defined in terms of a specific signal. The identification of definitive receptors for some of the plant hormones – for example, the ETR-like family of proteins as ethylene receptors – has afforded the possibility of quantifying sensitivity in terms of receptor abundance. Nevertheless, researchers who have attempted such exercises are still bound to equate such values with the extent of the physiological response (Klee, 2002).

## 2

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# Hormones and Signals: Identification and Description of Signalling Molecules

As a first step in developing the target cell concept for plants it is important that the major players in the known informational and signal repertoire are set out briefly at the start of the discussions. There are five major players: auxin, gibberellins, cytokinins, ethylene (and its precursor 1-aminocyclopropane-1-carboxylic acid, ACC), and abscisic acid. The first hormone to be discovered and isolated, auxin, is the best understood, the most important and without doubt the most remarkable. As well, the more recent signal molecules to be discovered are described in greater detail for some of them link more closely to molecules in the animal kingdom.

### Auxin

Indole-3-acetic acid (IAA) is the most abundant naturally occurring auxin, with indole-3-butyric acid (IBA) and 4-chloroindole-3-acetic acid (4-Cl-IAA) also occurring naturally (Figure 2.1). IAA was discovered in 1928 by Frits Went (Went, 1928) in the search for the chemical substance that was transported from the apex of the oat coleoptile and caused the cells below to elongate. In higher plants, several pathways of synthesis are possible. IAA is an indole derivative, and both *in vivo* and *in vitro* evidence indicates routes of synthesis from the aromatic amino acid, tryptophan, although more recent genetic and biochemical experiments have suggested that tryptophan-independent pathways may also operate to yield the final product (Bartel, 1997).

### *Auxin biosynthesis*

Tryptophan was proposed originally as the precursor of IAA due to structural similarities of the two molecules and when a clearly defined conversion was identified

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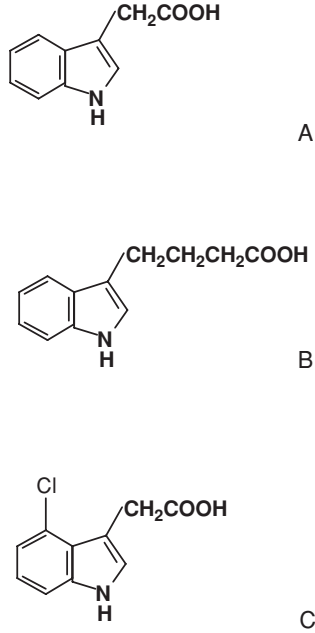
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## DESCRIPTION OF SIGNALS 7



**Figure 2.1.** Structures of naturally-occurring auxins: indole-3-acetic acid (A), indole-3-butyric acid (B), and 4-chloroindole-3-acetic acid (C).

in plant-associated microbes. Further, labelling studies *in vivo* in many plant species have shown that tryptophan can be metabolised to IAA (Normanly et al., 1995). However, the efficiency of this conversion to IAA was either not tested or shown to be very low, a concern when set against the background observation that tryptophan is readily converted to IAA non-enzymatically.

To resolve the role of tryptophan as the precursor of IAA, Wright et al. (1991) used the *orange pericarp* mutant of maize, a tryptophan auxotroph. This mutant arises from lesions in two unlinked loci of the tryptophan synthase B gene, and the total IAA produced in aseptically grown plants was found to be 50-fold greater than in normal maize seedlings. When aseptically grown mutant plants were labelled with [<sup>15</sup>N]-anthranilate, a tryptophan precursor, IAA was more enriched than tryptophan, leading the authors to conclude that IAA can be produced *de novo* without tryptophan as an intermediate. Further studies with other mutants have localised indole, another intermediate on the tryptophan pathway from anthranilate, as an IAA precursor, although some workers still challenge the validity of the tryptophan-dependent pathway (Muller and Weiler, 2000).

Nonetheless, there is increasing evidence to support tryptophan as the IAA precursor, although no pathway arising from tryptophan has been definitively established. Currently, three routes are now considered: the indole-3-pyruvate pathway, the tryptamine pathway and the indole-3-acetonitrile (IAN) pathway, although the IAN pathway appears to be restricted primarily to the Brassicaceae (Normanly et al., 1995). The conversion of tryptophan to indole-3-acetamide and then to IAA is most likely attributable to plant-associated microbes.

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## 8 HORMONES, SIGNALS AND TARGET CELLS

The recent characterisation of two cytochrome P450s, designated CYP79B2 and CYP79B3, that catalyse the formation of indole-3-acetaldoxime has created further interest in the IAN pathway (Hull et al., 2000; Zhao et al., 2002b). Indole-3-acetaldoxime can be converted to IAN and then to IAA, or to indole-glucosinilates creating a metabolic branch point. Nevertheless, the identification of a nitrilase gene that can convert IAN to IAA (Bartling et al., 1992) does support this route of IAA biosynthesis, although it appears to be restricted to only certain plant families.

The tryptamine biosynthetic route has also received support recently with the identification of the *YUCCA* gene from *Arabidopsis*, the product of which is a flavin monooxygenase-like enzyme that has been shown to catalyse the conversion of tryptamine to *N*-hydroxyl-tryptamine (Zhao et al., 2001).

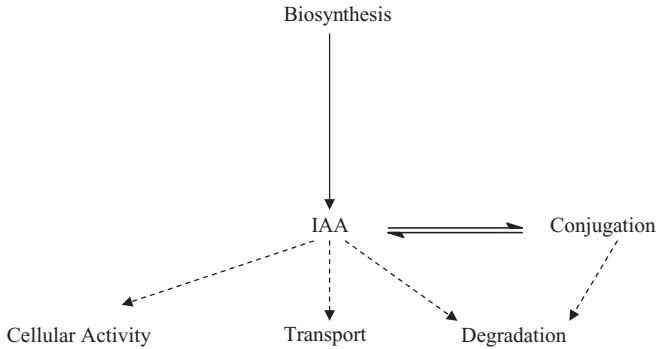
Such studies suggest that more than one pathway for IAA formation exists in plants, and further that more than one pathway may operate in the same tissues. An emerging consensus appears to be that tissues that produce transient, high levels of IAA utilise a version of the tryptophan-dependent pathway, while a tryptophan-independent pathway may supply the lower levels of IAA required for the maintenance of growth (Normanly and Bartel, 1999; Sztein et al., 2002). For readable accounts of the issues emerging in auxin biosynthesis, the reader is referred to the reviews of Normanly and Bartel (1999) and Bartel et al. (2001).

### *Auxin conjugation*

Of perhaps further significance is the extent of the conjugation of IAA, and its role in the regulation of IAA homeostasis in cells and tissues. The auxin-like biological activity of IAA-conjugates has been known for many years and these compounds have been proposed as slow release forms of free IAA (Hangarter and Good, 1981) to support the 'IAA homeostatic model' (Cohen and Bandurski, 1982) (Figure 2.2). The regulation of such slow release forms in any particular cell indicates how significant the enzymes and their genes can be in the developmental context of target cells. It is now widely appreciated that the formation of IAA-aspartate represents an irreversible conversion of IAA that marks the conjugate for eventual degradation (Monteiro et al., 1988). However, enzyme activities that can hydrolyse IAA conjugates back to free IAA occur in a number of species (Ludwig-Muller et al., 1996) and the cloning of genes coding for these hydrolases have shown that their expression is tightly developmentally regulated (Bartel and Fink, 1995; Davies et al., 1999; LeClere et al., 2002) demonstrating the tissue-specific location of IAA conjugates that have been identified in plants (Kowalczyk and Sandberg, 2001). For the target cell concept, the developmental regulation of free IAA release from conjugates provides a myriad of control points by which a competent auxin-responding cell can perceive differences in the levels of the hormone released.



## DESCRIPTION OF SIGNALS 9



**Figure 2.2.** Diagrammatic representation of the 'IAA homeostatic model' in which the many regulators of IAA concentration in plant cells are indicated.

### *Sites of auxin biosynthesis and transport*

In a careful study of the sites of auxin biosynthesis in *Arabidopsis*, the youngest leaves were found to contain the highest endogenous levels of IAA and also the highest capacity to synthesise the hormone (Ljung et al., 2001). A correlation of high IAA content and high rates of cell division has also been shown in developing tobacco leaves.

The importance of auxin in both cell-to-cell and long-distance signalling cannot be overestimated and the role of young shoots as sites of highest auxin concentration, cell division and highest rates of synthesis is critical. Because auxin can be stored in cells and tissues in biologically inactive forms such as amino acid conjugates and sugar esters, and then released again as the active auxin molecule (as in the gravity-stimulated nodes of grasses, or in germinating seeds), many cells have the potential to become sites of free IAA formation. In meristem parenchyma and the living non-vascular tissues of cortex or pith, auxin movement is from cell to cell, by a metabolic system unique to this molecule and its synthetic analogues. Remarkable amongst hormonal substances, auxin molecules are transported from the apical to basal end of each cell with respect to the shoot apical meristem; this polarity is continuous throughout the plant, progressing to the root apex where auxin efflux has been recorded. In tissue with a vascular supply, the transport of auxin is additionally served by the connection to both xylem and phloem. In immature tissue, below an apical meristem, the basipetal polarity of auxin movement is high; but as cells reach maximum size and mature, this differential between downward (polar) and upward (diffusion) movement becomes barely detectable as determined by auxin transport assays (Goldsmith, 1977). The major transport pathways then reside in the vascular tissues, which are predominantly long distance. Both long- and short-distance transport pathways therefore operate and coordinate an auxin-signalling mechanism between all parts of the plant and throughout the plant's developmental lifetime (see Chapter 3 for a description of auxin transport).

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## 10 HORMONES, SIGNALS AND TARGET CELLS

Whereas auxin is a major hormone in promoting cell expansion and elongation growth, additions of auxin do not cause mature cells to enlarge further. This does not mean that auxin is no longer a regulator of cell performance, but rather that the role of auxin in gene expression changes as the cell grows older. Instead of regulating events of cell size, auxin levels then determine the entry into flowering, fruit ripening, senescence and abscission, all being essentially terminal stages of cell differentiation. The cell has changed from its original target status, although the signal molecule, auxin, has remained the same.

### Gibberellins

This group of hormones was isolated and characterised following a study of interspecies signals. A fungal disease of rice, common in Japan, was the cause of abnormally high growth of the plant associated with yellowing leaves and wilting. In 1926, Eishii Kurosawa added a culture filtrate of the fungus to normal rice plants and demonstrated that a substance present in this filtrate led to the enhanced cell elongation that resulted in extra tall plants (Kurosawa, 1926). This, and the even later isolation of  $GA_A$  and  $GA_B$  by Yabata and Sumiki (1938), was published in Japanese and remained untranslated until after World War II. The knowledge that auxin analogues could be used as selective herbicides led to a crescendo of research in the West to discover more natural growth-regulating compounds in plants. The Japanese literature then revealed the potential of the family of gibberellin-related substances, and their presence in higher plants as well as in fungi. Although present in all plants, gibberellins are unlike auxin in not being transported in a polar way, nor are they involved in phototropic or gravity-induced curvatures of plant parts. Of special significance is their ability to induce flowering in long-day plants held under non-inductive short-day conditions (see Zeevaart, 1976). This signal to flower is not, however, necessarily applicable to short-day plants held under non-inductive daylengths, so gibberellins cannot properly be considered as flowering hormones. Also, gibberellins can direct the apices of plants with unisexual flowers (for example, Cucurbits) to the production of predominantly staminate expression, while auxin treatment favours the formation of female flowers. Here then, was the second major discovery of a family of signalling molecules. But whereas auxin seemed essential and universal to the growth of plants, as Frits Went wrote “Ohne Wuchsstoff, kein Wachstum” (Went, 1928), gibberellins showed much greater specificity – the cell growth enhancement response, for example, was greatest in genetic dwarfs and almost absent in the normal wild-type (Phinney, 1956). For gibberellins, therefore, the target status of the cell was recognised early on as genetically as well as developmentally determined.

### *Gibberellin biosynthesis*

Structurally, gibberellins are all sesquiterpenes, and they exist in plant tissues as the  $C_{20}$ - or  $C_{19}$ -GAs (Figure 2.3). These structures are derived from