

What's New in Plant Physiology

Hormone trafficking. A case study of growth regulator dynamics

Thomas G. Brock

Brock, T. G. 1993. Hormone trafficking. A case study of growth regulator dynamics. – *Physiol. Plant.* 89: 237–241.

When growth is promoted in plants, endogenous hormones may move into or out of the growing tissue, change in concentration through synthesis or degradation, or be chemically converted between active and inactive forms. Furthermore, the sensitivity of the growing tissue itself may change, producing an effective change in hormone level. The complexity of hormone trafficking has been partially unraveled in the case of the leaf-sheath pulvinus of grasses. In this specialized organ, which grows only when turned on its side, hormones including auxins, gibberellins and ethylene change dynamically through time and space to regulate growth.

Key words – Auxin, ethylene, gibberellin, growth.

T. G. Brock, 3317 Med. Sci. I, Univ. of Michigan, Ann Arbor, MI 48109-0652, USA.

Introduction

Plant hormones modulate many plant processes, and each plant process may be regulated by several phytohormones. This complexity of regulation is a significant challenge to researchers who study hormonally-regulated processes. The challenge is further complicated by ongoing changes in hormone dynamics during the response: each hormone involved in a given process can be synthesized or destroyed, chemically altered to change activity at various times before or during the response, or physically shuttled from one location to another. Furthermore, the responsiveness of the organ to each hormone can change, producing the appearance of a change in hormone level. These changes in hormone dynamics, or 'hormone trafficking', are the subject of this review.

An excellent model system for the study of hormone trafficking during the process of growth is the leaf-sheath pulvinus of grasses (for review, see Kaufman and Brock 1992). This organ is a swollen region found at the base of each leaf sheath of the grass stem, at the 'joint' region (Fig. 1a). Its primary function is to keep the plant tip upright. As long as the pulvinus itself remains vertical, it is non-growing. When the grass stem leans for a prolonged period (i.e. when it receives a 'gravistimulus'), the pulvinus responds by growing asymmetri-

cally (Fig. 1b), bringing the plant apex back to a vertical orientation. Unlike the motor response of the classical pulvinus, e.g. of legumes, this response is a true growth response and is *not* reversible.

Three features of the leaf-sheath pulvinus make it ideal for the study of hormone trafficking. First, the growth response is an 'on/off' response: without the gravistimulus, the pulvinus is non-growing; when the stem leans, growth is initiated in an asymmetric fashion. Second, at least three classes of hormones are known to be involved in growth regulation: auxins, gibberellins and ethylene (Kaufman et al. 1976, 1985, Wright et al. 1978). Finally, the response is slow and prolonged, allowing for better understanding of the kinetics of action of each element in the system.

A great deal is known about the regulation of growth in the leaf-sheath pulvinus. A general scheme of how some of the key elements interact is shown in Fig. 2. Hormones are presented in a 'traditional' manner, at the top of a cascade of changes which ultimately affect growth. In fact, components of the cascade can feed back to alter the trafficking of the original hormone, as well as influence other hormones in the same system. Furthermore, different hormones may enter into the cascade at different points. Some of these complexities add to the intrigue of hormone trafficking and will be considered below.

Received 27 April, 1993

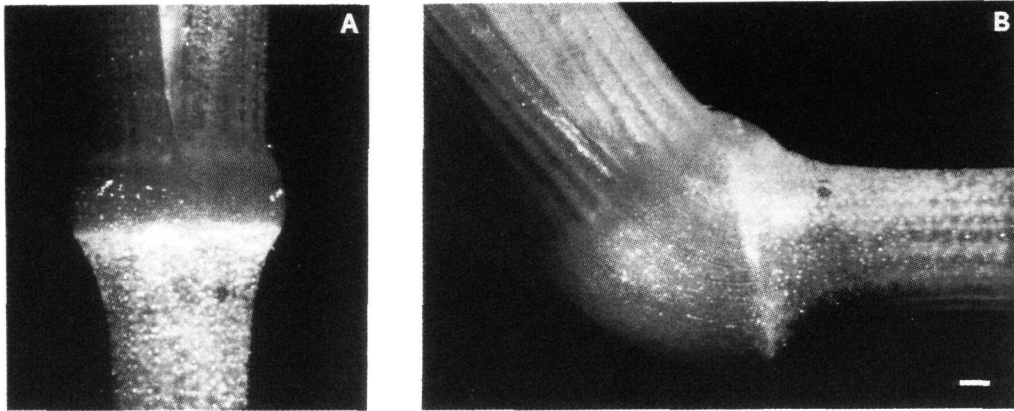


Fig. 1. The leaf-sheath pulvinus from upright (A) vs horizontally-placed (B) oat plants. The gravistimulated pulvinus has been in the horizontal position for 48 h and has nearly completed its response. Note that the response does not form a right angle: two or more pulvini must work together to bring the shoot apex back to a vertical orientation. Scale bar = 1 mm.

Translocation of hormones within the pulvinus

Movement within the pulvinus before and during the growth response is an important aspect of the action of each hormone. The pattern of growth also reveals something about where the hormones are working. Treatment of barley pulvini with coumarin, an inhibitor of cellulose synthesis, reorients the direction of cell growth from longitudinal to transverse, so the pattern of response is visible in cross section (Dayanandan and Kaufman 1984). This pattern of response is a continuous gradient of increasing growth from the uppermost cell to the lowermost in the horizontally-placed pulvinus (Brock et al. 1989). Such a gradient in response suggests a gradient in hormone, possibly through movement of individual molecules.

The movement of the three types of hormones, auxin, gibberellins and ethylene, appears to be characteristic for that hormone. We know the most about auxin translocation. In the upright, non-growing pulvinus, auxin (indole-3-acetic acid, or IAA) enters the pulvinus from

the leaf above, moving via the energy-dependent polar auxin transport system (Wright 1981). If the pulvinus remains upright, most of the auxin continues through the pulvinus and is exported into the node below. However, if the stem is turned on its side, the direction of transport changes. Export ceases (Harrison and Kaufman 1980). IAA moves to the lower half of horizontal pulvini (Merkis and Novitskene 1969, Wright 1986), or reverses its direction in inverted pulvini (Wright 1982). In horizontal pulvini, translocation is quite rapid and precedes the asymmetric growth response. Addition of sucrose speeds up the rate of translocation and also reduces the lag to the growth response (Brock et al. 1991). This indicates that lateral transport of IAA across the pulvinus is involved in initiating growth, with higher growth rates reflecting higher amounts of IAA. However, treatment with inhibitors of polar auxin transport block IAA translocation completely, but only reduce growth by 50% (Brock et al. 1991). Hence, IAA translocation alone cannot be the critical process regulating growth.

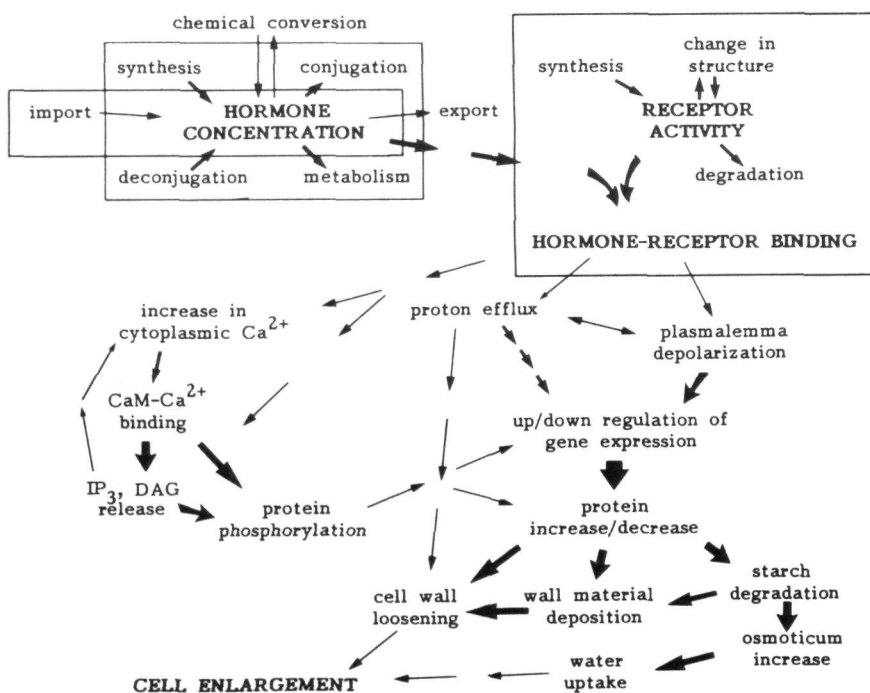


Fig. 2. A simplified scheme of the role of hormone trafficking in the regulation of growth in the leaf-sheath pulvinus. The system becomes more complicated when possible feedback loops from the response cascade to 'hormone levels' are included. Also, several hormones may feed into the growth cascade at different points and at different times.

Gibberellins also move within the pulvinus like IAA, redistributing from the upper side of horizontal pulvini and accumulating in the lower side (Rood et al. 1987). However, this translocation of gibberellins does not appear to be as rapid as that for IAA.

Another type of gibberellin translocation is also important during the graviresponse: movement across the plasmalemma and into the cytosol is strongly enhanced. In the upright, non-growing pulvinus, gibberellins move very slowly, if at all, across the plasmalemma (Kaufman et al. 1985, Rood et al. 1987). Horizontal placement of the pulvinus initiates growth and strongly promotes uptake of gibberellins (Pharis et al. 1981, Rood et al. 1987). This process moves the hormone from a site where it cannot act to a site where it can bind to its receptor and initiate processes leading to growth enhancement. In this case, translocation appears to be relatively slow, suggesting that the role of gibberellin is in maintaining the growth response, rather than initiating it.

Ethylene movement is characteristic for the hormone. Since ethylene is a gas, it moves in a relatively non-directional manner. Instead, it is spread throughout the pulvinus. The timing of its release may reflect its role. Ethylene is not released in measurable quantities until several hours after the initiation of growth in the horizontal pulvinus, and growth rate begins to diminish after the release of ethylene (Kaufman et al. 1985). As a result, it seems plausible that ethylene is involved in stopping the growth response.

Direct synthesis of hormones within the pulvinus

An obvious method of increasing hormone levels is through synthesis. There is excellent evidence that active forms of all three types of hormones increase in amount in the pulvinus following stimulation. However, it appears unlikely that either auxins or gibberellins are synthesized extensively from building block precursors within the pulvinus as a response to gravistimulation. Instead, their amounts increase primarily by altered transport, as discussed above, or by chemical conversion from inactive forms, as presented later.

Ethylene, in contrast, is thought to increase exclusively through de novo synthesis (Kaufman et al. 1985). Furthermore, its production is assumed to be promoted by auxin. One scenario might be that altered transport of auxin in the horizontal pulvinus leads to a gradual buildup of auxin on the lower side. This regional excess of auxin then initiates the localized synthesis of ethylene. Ethylene then spreads from the site of synthesis, producing a number of biochemical effects which translate to a slowing, and, ultimately, a stopping of growth.

The chemical conversion of hormone forms

One of the most intriguing, but often overlooked, aspects of growth regulator dynamics is the interconver-

sion of hormones between active and inactive forms. This process, also referred to as conjugation/deconjugation, should be particularly effective in responses where synthesis or import of hormones may be too slow or otherwise impractical.

The pulvinus in the upright grass shoot may be viewed as an organ waiting for the proper stimulus. It is positioned on the shoot between the leaf and the rest of the plant. As a result, leaf products (e.g. photosynthate, hormones) are moving down through the pulvinus while leaf needs (e.g. water, nutrients) course up through it. Large amounts of carbohydrates, arriving from the leaf, are retained by the pulvinus, primarily in the form of starch grains (Song et al. 1988). Some of these carbohydrates will be used to drive the graviresponse (Bridges and Wilkins 1974). Similarly, large amounts of hormones are stored within the pulvinus in inactive forms. Some of these will be converted to active forms to regulate the growth response. Hence, the upright pulvinus is a warehouse of the components necessary for the pulvinus' main function of responding to the gravistimulus.

Auxin is stored in two forms in the upright pulvinus. An amide-linked conjugate predominates, but an ester-linked conjugate is also found in significant amounts (P. B. Kaufman, personal communication). When the pulvinus is gravistimulated, both types of conjugated IAA decrease and the amount of free IAA increases (Kaufman et al. 1987). For auxin, production of inactive forms predominates before gravistimulation. Upon gravistimulation, production of inactive forms is minimized throughout the pulvinus, and inactive forms are converted to the active form, IAA.

Following gravistimulation, much more IAA is found in the lower part of the pulvinus than in the upper half (Wright et al. 1978, Wright 1986). However, it is unclear whether this results from asymmetric conversion of inactive IAA to free IAA, as has been proposed (Kaufman et al. 1987), or to lateral transport of free IAA following symmetrical conversion. Also, the total amount of free IAA found in pulvini following gravistimulation greatly exceeds the total conjugated and unconjugated IAA found in the upright pulvinus (Wright 1986, Kaufman et al. 1987). While some of this increase in IAA may be from de novo synthesis, the majority probably comes from import from the leaf, since this process continues in the horizontal pulvinus (Brock et al. 1991).

Gibberellin interconversion is a hallmark of the hormone's mechanism of action (Brock and Kaufman 1991), and this is certainly true in the pulvinus. As for IAA, inactive gibberellin molecules are chemically converted into active forms in the lower, growing halves of horizontal pulvini (Pharis et al. 1981, Rood et al. 1987). Furthermore, in the upper halves, where growth is minimized, inactive forms are accumulated much more than are active forms. This suggests that there is differential interconversion between active and inactive forms fol-

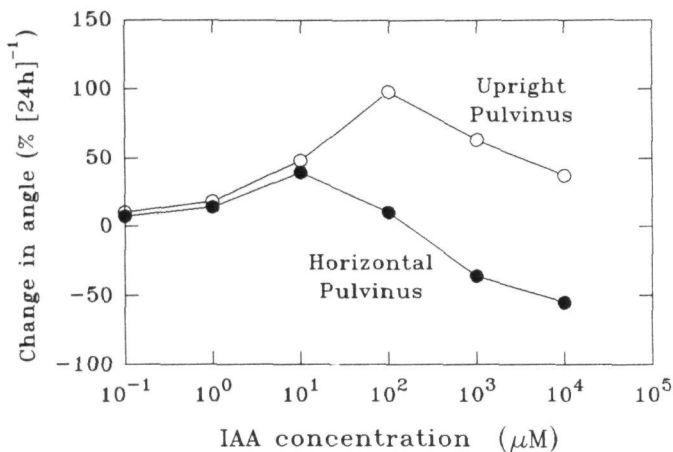


Fig. 3. The effect of unilaterally-applied IAA on pulvinus growth in upright vs horizontal stems. IAA was prepared in agar blocks and placed against one side of upright pulvini, or against the lower side of horizontal pulvini. After 24 h, the change in angle through the pulvinus was measured. Results are presented as the difference between hormonally-treated pulvini and pulvini placed similarly without hormone. Modified after Brock and Kaufman 1988.

lowing gravistimulation. In the uppermost, slow-growing cells, active gibberellins may become conjugated and thus inactivated. In the lower, rapidly growing cells, conjugated gibberellins may be converted to active forms. Alternatively, there may be differential transport, with active forms moving downward in the horizontal pulvinus and inactive conjugates moving upward.

Changes in responsiveness to hormones

In many circumstances, a stimulus will produce a change in the plant that alters the plant's responsiveness to a given amount of hormone. There are two ways that such changes pertain to hormone trafficking. First, the change could be due to a direct effect on how the hormone and its receptor interact (e.g. enhanced binding of the hormone to the receptor). In this case, there is a *true* increase in the hormone activity and a true change in sensitivity (see, e.g., Firn 1986). In a second scenario, increased responsiveness might result indirectly, for example, from an increase in carbohydrate availability for synthesis and energy during cell expansion. In this case, there is an *apparent*, or *effective*, increase in hormone activity. In both cases, the physiological changes are integral components of the hormone dynamics of the system.

Placing the pulvinus on its side changes the responsiveness of the pulvinus to both IAA and gibberellic acid (GA₃). For IAA, the gravistimulus has no effect on the amount of growth induced by low amounts of the hormone (Brock and Kaufman 1988). However, for higher doses of IAA, gravistimulation actually *decreases* the tissue responsiveness, and this effect becomes greater as the amount of free IAA increases (Fig. 3). This would tend to build a braking feedback loop into the system: the smaller amounts of IAA seen during the

earlier stages of response promote growth, but, as free IAA accumulates on lower sides through release from conjugate and redirected transport, growth would slow and eventually stop. Curiously, this decrease in responsiveness is much less evident on the upper side of horizontal pulvini. As a result, if IAA is delivered uniformly, for example from an agar block placed on the apex of the pulvinus, then elongation of the upper side can almost match that of the lower side.

The scenario for gibberellins is very different. Upright pulvini are only slightly responsive to GA₃. However, following gravistimulation, pulvini become much more responsive, with micromolar concentrations of GA₃ significantly enhancing the growth response (Brock and Kaufman 1988). Furthermore, this enhanced responsiveness occurs throughout the pulvinus, not just on the lowermost side. One explanation for the change in responsiveness to GA₃ is the enhanced uptake of gibberellins induced by the gravistimulus, as described earlier. The evidence indicates that the stimulus induces the uptake of all molecular forms of gibberellins, and then differential metabolism and enhanced transport follow.

Future directions

Most contemporary research, in most phases of hormonal regulation of growth, addresses the *initiation* of growth. Few studies pursue hormone dynamics during growth maintenance, and fewer still consider the cessation of growth. Does growth stop because active forms of the hormone have left the tissue? Or has the sensitivity of the tissue diminished below some essential threshold? How are high growth rates maintained, or why do they fail to be maintained? Similarly, how long do hormones continue to act during a response, and why do they cease to act? These questions are clearly relevant to agricultural and horticultural concerns.

Another area begging additional effort is hormone interaction. Few systems are influenced solely by a single regulator. More commonly, multiple hormones work in series or in parallel to initiate, then modify, and finally to terminate a response. When regulators work sequentially, the first in the series may influence the tissue response to subsequent regulators. When regulators work simultaneously, one may produce a change in one parameter (for example, enhanced synthesis of a product) which may be affected by the action of another (e.g. product utilization or export). These questions need to be addressed at the plant, cell and molecular levels.

Finally, the numerous types of regulators, in any given plant response, need to be clearly identified. There are many non-hormonal regulators which can feed into the response cascade. These may include endogenous chemical regulators, such as calcium or arachidonic acid, as well as exogenous chemicals. Also, environmental stimuli can control response cascades without

using hormonal mediators. Examples include heat shock, various light stimuli and gravity. How these non-hormonal factors interplay with hormones in controlling plant development is a major research problem for the future.

Acknowledgements – I thank Dr Peter B. Kaufman for support through the execution of this work, and Scott Holling and Lan Lan Lee for work on changing tissue sensitivity to exogenous hormones.

References

- Bridges, I. G. & Wilkins, M. B. 1974. The role of reducing sugars in the geotropic response of the wheat node. – *Planta* 117: 243–250.
- Brock, T. G. & Kaufman, P. B. 1988. Altered growth response to exogenous auxin and gibberellic acid by gravistimulation in pulvini of *Avena sativa*. – *Plant Physiol.* 87: 130–133.
- & Kaufman, P. B. 1991. Growth regulators: An account of hormones and growth regulation. – *In Plant Physiology, A Treatise, Vol. X: Growth and Development* (F. C. Steward and A. D. Krikorian, eds), pp. 277–340. Academic Press, Inc., New York, NY. ISBN 0-12-668610-6.
- , Lu, C. R., Ghosheh, N. S. & Kaufman, P. B. 1989. Localization and pattern of graviresponse across the pulvinus of barley (*Hordeum vulgare*). – *Plant Physiol.* 91: 744–748.
- , Kapek, E. H., Ghosheh, N. S. & Kaufman, P. B. 1991. Dynamics of auxin movement in the gravistimulated leaf-sheath pulvinus of oat (*Avena sativa*). – *J. Plant Physiol.* 138: 57–62.
- Dayanandan, P. & Kaufman, P. B. 1984. Analysis and significance of gravity-induced asymmetric growth in the grass leaf-sheath pulvinus. – *Ann. Bot.* 53: 29–44.
- Firn, R. D. 1986. Growth substance sensitivity: the need for clearer ideas, precise terms and purposeful experiments. – *Physiol. Plant.* 67: 267–272.
- Harrison, M. A. & Kaufman, P. B. 1980. Hormonal regulation of lateral bud (tiller) release in oats (*Avena sativa* L.). – *Plant Physiol.* 66: 1123–1127.
- Kaufman, P. B. & Brock, T. G. 1992. Structural development of the oat plant. – *In Oat Science and Technology* (H. Marshall and R. D. Sorrells, eds), pp. 53–75. American Society of Agronomy and Crop Science Society of America, Madison, WI. ISBN 0-89118-110-5.
- , Ghosheh, N. S., Nakosteen, L., Pharis, R. P., Durley, R. C. & Morf, W. 1976. Analysis of native gibberellins in the internode, nodes, leaves, and inflorescence of developing *Avena* plants. – *Plant Physiol.* 58: 131–134.
- , Pharis, R. P., Reid, D. M. & Beall, F. D. 1985. Investigations into the possible regulation of negative gravitropic curvatures in intact *Avena sativa* plants and in isolated stem segments by ethylene and gibberellin. – *Physiol. Plant.* 65: 237–244.
- , Brock, T. G., Song, I., Rho, Y. B. & Ghosheh, N. S. 1987. How cereal grass shoots perceive and respond to gravity. – *Am. J. Bot.* 74: 1446–1457.
- Merkis, A. I. & Novitskenskaya, L. L. 1969. The character of distribution of auxin in the stem nodes of graminaceous plants in connection with their lodging. – *Agrokhimizy* 10: 87–100.
- Pharis, R. P., Legge, R. L., Noma, M., Kaufman, P. B., Ghosheh, N. S., LaCroix, J. D. & Heller, K. 1981. Changes in endogenous gibberellins and the metabolism of [³H]-GA₄ after geostimulation in shoots of the oat plant (*Avena sativa*). – *Plant Physiol.* 67: 892–897.
- Rood, S. B., Kaufman, P. B., Abe, H. & Pharis, R. P. 1987. Gibberellins and gravitropism in maize shoots. Endogenous gibberellin-like substances and movement and metabolism of [³H]-gibberellin A₂₀. – *Plant Physiol.* 83: 645–651.
- Song, I., Lu, C., Brock, T. G. & Kaufman, P. B. 1988. Do starch statoliths act as the gravisensors in cereal grass pulvini? – *Plant Physiol.* 86: 1155–1162.
- Wright, M. 1981. Reversal of polarity of IAA transport in the leaf sheath base of *Echinochloa colonum*. – *J. Exp. Bot.* 32: 159–169.
- 1982. The polarity of movement of endogenously produced IAA in relation to a gravity perception mechanism. – *J. Exp. Bot.* 33: 929–934.
- 1986. The acquisition of gravisensitivity during the development of nodes of *Avena fatua*. – *J. Plant Growth Regul.* 5: 37–47.
- , Mousdale, D. M. A. & Osborne, D. J. 1978. Evidence for a gravity-regulated level of endogenous auxin controlling cell elongation and ethylene production during geotropic bending in grass nodes. – *Biochem. Physiol. Pflanz.* 172: 581–596.

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.